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Induction of Homologous Recombination in Mammalian Chromosomes by Using the I-*Sce*I System of *Saccharomyces cerevisiae*

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The mitochondrial intron-encoded endonuclease I-*Sce*I of *Saccharomyces cerevisiae* has an 18-bp recognition sequence and, therefore, has a very low probability of cutting DNA, even within large genomes. We demonstrate that double-strand breaks can be initiated by the I-*Sce*I endonuclease at a predetermined location in the mouse genome and that the breaks can be repaired with a donor molecule homologous with regions flanking the breaks. This induced homologous recombination is approximately 2 orders of magnitude more frequent than spontaneous homologous recombination and at least 10 times more frequent than random integration near an active promoter. As a consequence of induced homologous recombination, a heterologous novel sequence can be inserted at the site of the break. This recombination can occur at a variety of chromosomal targets in differentiated and multipotential cells. These results demonstrate homologous recombination involving chromosomal DNA by the double-strand break repair mechanism in mammals and show the usefulness of very rare cutter endonucleases, such as I-*Sce*I, for designing genome rearrangements.

Homologous recombination (HR) between chromosomal and exogenous DNAs is at the basis of methods for introducing genetic changes into the genome (5, 20). Parameters of the recombination mechanism have been determined by studying plasmid sequences introduced into cells (1, 4, 10, 11) and in an in vitro system (8). HR is inefficient in mammalian cells but is promoted by double-strand breaks in DNA.

So far, it has not been possible to cleave a specific chromosomal target efficiently, thus limiting our understanding of recombination and its exploitation. Among endonucleases, the *Saccharomyces cerevisiae* mitochondrial endonuclease I-*Sce*I (6) has characteristics which can be exploited as a tool for cleaving a specific chromosomal target and, therefore, manipulating the chromosome in living organisms. I-*Sce*I protein is an endonuclease responsible for intron homing in yeast mitochondria, a nonreciprocal mechanism by which a predetermined sequence becomes inserted at a predetermined site. It has been established that the I-*Sce*I endonuclease can induce recombination in yeast nuclei (16) and can enhance extrachromosomal recombination in COS-1 cells (17) by initiating a double-strand break. The recognition site of the I-*Sce*I endonuclease is 18 bp long; therefore, I-*Sce*I recognition sequences are very rare in genomic DNA (22). In addition, as the I-*Sce*I protein is not a recombinase, its potential for chromosome engineering is larger than that of systems with target site requirements on both host and donor molecules (9).

We demonstrate here that the yeast I-*Sce*I endonuclease can efficiently induce double-strand breaks in a chromosomal target in mammalian cells and that the breaks can be repaired with a donor molecule homologous with the regions flanking the break. The enzyme catalyzes recombination at a high efficiency. This demonstrates that recombination between chromosomal DNA and exogenous DNA can occur in mammalian cells by the double-strand break repair mechanism (19).

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MATERIALS AND METHODS

Plasmid construction. pG-MPL was obtained in four steps: (i) insertion of the 0.3-kbp *Bgl*II-*Sma*I fragment (treated with Klenow enzyme) of the Moloney murine leukemia virus *env* gene (25) containing a splice acceptor site (SA) between the *Nhe*I and *Xba*I sites (treated with Klenow enzyme), in the U3 sequence of the 3′ long terminal repeat (LTR) of Moloney murine leukemia virus, in an intermediate plasmid, (ii) insertion in this modified LTR with linker adaptors of the 3.5-kbp *Nco*I-*Xho*I fragment containing the *Phleo-lacZ* fusion gene (14) (from pUT65 from the Cayla laboratory) at the *Xba*I site next to SA, (iii) insertion of this 3′ LTR (containing the SA and *Phleo-lacZ*), recovered by *Sal*I-*Eco*RI double digestion in the p5′LTR plasmid (a plasmid containing the 5′ LTR fused to nucleotide 563 of Moloney murine leukemia virus (25) between the *Xho*I and the *Eco*RI sites, and (iv) insertion of a synthetic I-*Sce*I recognition site into the *Nco*I site in the 3′ LTR (between the SA and *Phleo-lacZ*) (Fig. 1A). pG-MtkPL was obtained by insertion (antisense to the retroviral genome) of the 1.6-kbp *tk* gene with its promoter with linker adaptors at the *Pst*I site of pG-MPL (Fig. 1A).

pVRneo was obtained in two steps: (i) insertion into pSP65 (from Promega) linearized by *Pst*I-*Eco*RI double digestion of the 4.5-kbp *Pst*I-to-*Eco*RI fragment of pG-MPL containing the 3′ LTR with the SA and *Phleo-lacZ* and (ii) insertion of the 2.0-kbp *Bgl*II-*Bam*HI fragment (treated with Klenow enzyme) containing *neo* poly(A) from pRSVneo into the *Nco*I restriction site of the 3′ LTR of pG-MPL (Fig. 1B).

pCMV(I-*Sce*I+) was obtained in two steps: (i) insertion of the 0.73-kbp *Bam*HI-*Sal*I, I-*Sce*I-containing fragment (from pSCM525 [A. Thierry; personal gift]) into the phCMV1 plasmid (F. Meyer; personal gift) cleaved at the *Bam*HI and the *Sal*I sites and (ii) insertion of a 1.6-kbp fragment (nucleotides 3204 to 1988 in simian virus 40) containing the polyadenylation signal of simian virus 40 into the *Pst*I site of phCMV1 (Fig. 1C). pCMV(I-*Sce*I−) contains the I-*Sce*I open reading frame (ORF) in reverse orientation compared with the pCMV(I-*Sce*I+) plasmid. It was obtained by inserting the *Bam*HI-*Pst*II-*Sce*I ORF of the pCMV (I-*Sce*I+) fragment (treated with Klenow enzyme) into an intermediate plasmid, the phCMV poly(A) vector linearized by *Nsi*I and *Sal*I double digestion and treated with Klenow enzyme (Fig. 1C).

Cell culture and selection. NIH 3T3, PCC7-S, and ψ-2 cells are described in references 7 and 12. For selection, ganciclovir (13, 23) was added in the tissue culture medium at a concentration of 2 μM. Ganciclovir selection was maintained on cells during 6 days. G418 was added into the appropriate medium at a concentration of 1 mg/ml for PCC7-S cells and 400 μg/ml for NIH 3T3 cells. Phleomycin was used at a concentration of 10 μg/ml.

Transfection, infection, cell staining, and nucleic acid blot analysis. Transfection, infection, cell staining, and nucleic acid blot analysis were performed as described in references 2 and 3.

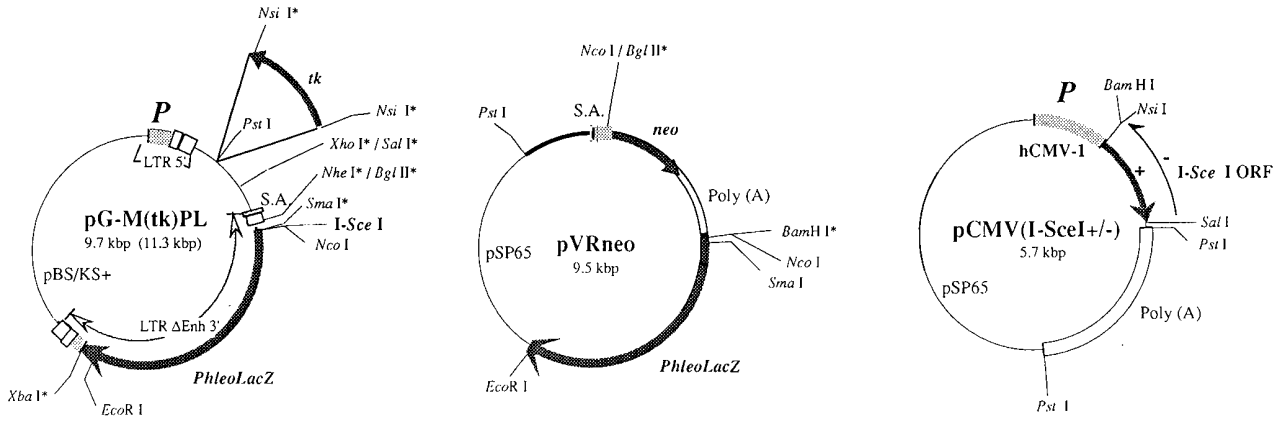

FIG. 1. Plasmids used in this study. (A) Diagram of the 9.7-kbp pG-MPL plasmid and the 11.3-kbp pG-MtkPL plasmid. pG-MtkPL is the product of the insertion of the 1.6-kbp *Nsi*I fragment containing the *tk* gene at the *Pst*I site of pG-MPL (the ORF of the *tk* gene is in reverse orientation relative to the *Phleo-lacZ* gene). (B) Diagram of the 9.5-kbp pVRneo plasmid. (C) Diagram of the 5.7-kbp pCMV(I-*Sce*I+) and the 5.7-kbp pCMV(I-*Sce*I−) plasmids. The pCMV(I-*Sce*I−) plasmid has the I-*Sce*I ORF in the reverse orientation relative to the human cytomegalovirus 1 promoter. *P*, eucaryotic promoter. Genes to be expressed in eucaryotic cells are represented by filled bars. Arrows, ORF orientation; asterisks, restriction enzyme recognition sites destroyed by cloning.

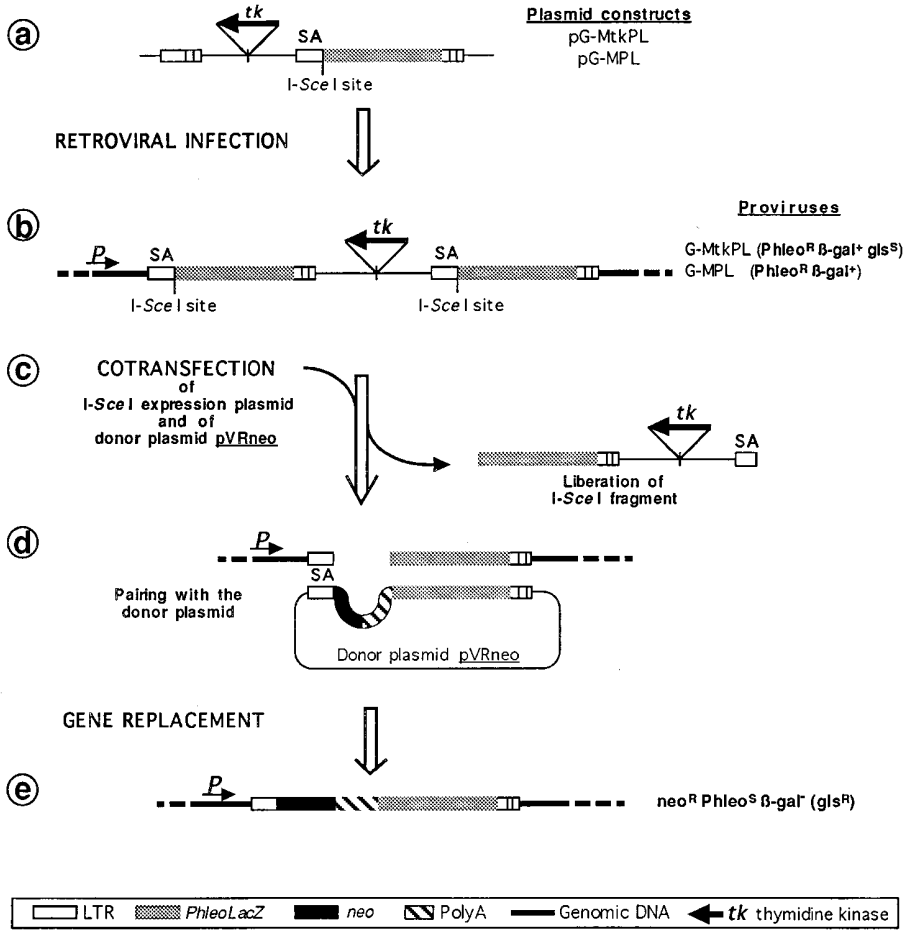

FIG. 2. Experimental design for the detection of HR induced by I-*Sce*I. (a) Map of the 7.5-kbp *tk-Phleo-lacZ* retrovirus (G-MtkPL) and the 6.0-kbp *Phleo-lacZ* retrovirus (G-MPL). G-MtkPL sequences (from G-MtkPL virus) contain a *Phleo-lacZ* fusion gene for positive selection of infected cells (in phleomycin-containing medium) and a *tk* gene for negative selection (in ganciclovir-containing medium). G-MPL sequences (from G-MPL virus) contain only *Phleo-lacZ* sequences. (b) Map of proviral structures following retroviral integration of G-MtkPL and G-MPL. I-*Sce*I *Phleo-lacZ* LTR duplicates, placing I-*Sce*I *Phleo-lacZ* sequences in the 5′ LTR. The virus vector (which functions as a promoter trap) is transcribed (arrow) by a flanking cellular promoter, *P*. (c) I-*Sce*I creates two double-strand breaks in the host DNA, liberating the central segment and leaving broken chromosome ends that can pair with the donor plasmid, pVRneo (d). (e) Expected recombinant locus following HR.

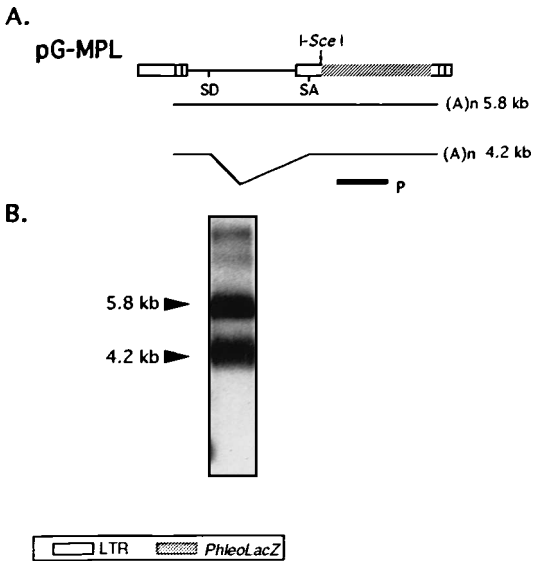

FIG. 3. (A) Diagram of pG-MPL. SD, splice donor site. The structures of the unspliced 5.8-kb (genomic) and spliced 4.2-kb transcripts are shown below. P, ^{32}P-radiolabelled *lacZ* probe. (B) Northern blot analysis of a pG-MLP-transformed ψ-2 producer clone using polyadenylated RNA. Note that the genomic and the spliced mRNAs are produced at the same high level.

RESULTS

To detect I-*Sce*I HR, we have designed the experimental system shown in Fig. 2. Defective recombinant retroviruses (24) were constructed with the I-*Sce*I recognition site and a *Phleo-lacZ* (14) fusion gene inserted in their 3′ LTRs (Fig. 2a). Retroviral integration into the cell genome results in two I-*Sce*I sites 5.8 kbp from each other for G-MPL and 7.2-kbp from each other for G-MtkPL (Fig. 2b). We hypothesized that I-*Sce*I-induced double-strand breaks at these sites (Fig. 2c) could initiate HR with a donor plasmid (pVRneo; Fig. 2d) containing sequences homologous to the flanking regions of the double-strand breaks and that nonhomologous sequences, carried by the donor plasmid, could be copied during this recombination (Fig. 2e).

Introduction of duplicated I-*Sce*I recognition sites into the genomes of mammalian cells by retrovirus integration. Two proviral sequences were used in these studies. The G-MtkPL proviral sequences (from G-MtkPL virus) contain the *Phleo-lacZ* fusion gene for positive selection of transduced cells (in phleomycin-containing medium) and the *tk* gene for negative selection (in ganciclovir-containing medium). The G-MPL proviral sequences (from G-MPL virus) contain only the *Phleo-lacZ* sequences. G-MtkPL and G-MPL are defective recombinant retroviruses (15) constructed from an enhancerless Moloney murine leukemia provirus. The virus vector functions as a promoter trap and, therefore, is activated by flanking cellular promoters.

Virus-producing cell lines were generated by transfecting pG-MtkPL or pG-MPL into the ψ-2 packaging cell line (13). Northern (RNA) blot analysis of viral transcripts shows that the ψ-2-G-MPL line expressed 4.2- and 5.8-kb transcripts that hybridized with *lacZ* probes (Fig. 3). These transcripts probably initiate in the 5′ LTR and terminate in the 3′ LTR. The 4.5-kb transcript corresponds to the spliced message, and the 5.8-kb transcripts correspond to the unspliced genomic message (Fig. 3A). This verified the functionality of the 5′ LTR and of the splice donor and acceptor in the virus (Fig. 3B).

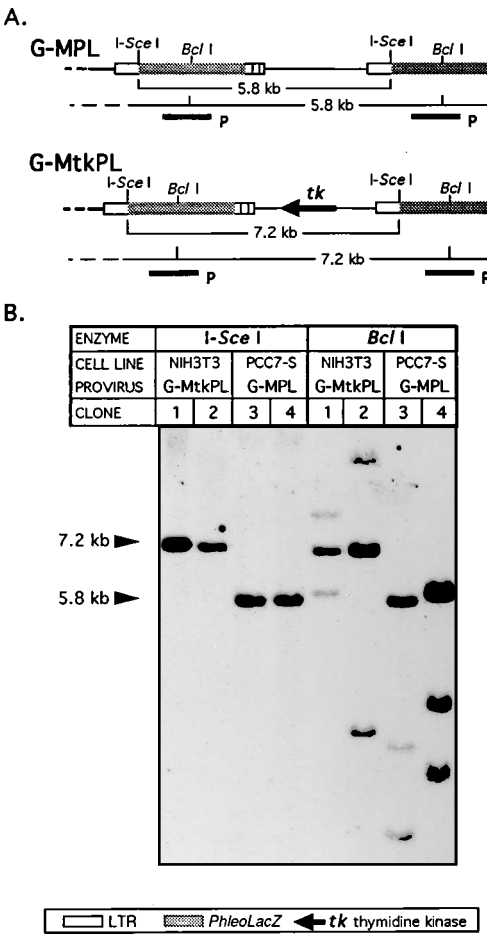

FIG. 4. (A) Introduction of duplicated I-*Sce*I recognition sites into the genomes of mammalian cells by retrovirus integration. G-MPL and G-MtkPL proviruses are diagrammed, and the positions of the two LTRs and pertinent restriction sites are indicated. The sizes of *Bcl*I fragments and I-*Sce*I fragments are indicated. P, ^{32}P-radiolabelled *lacZ* probe. (B) Southern blot analysis of cellular DNAs from NIH 3T3 fibroblasts infected by G-MtkPL and PCC7-S multipotent cells infected by G-MPL, with *Bcl*I digests demonstrating LTR-mediated *Phleo-lacZ* duplication and I-*Sce*I digests demonstrating faithful duplication of I-*Sce*I sites.

Similar results have been obtained with ψ-2-G-MtkPL. Virus was prepared from the culture medium of ψ-2 cell lines.

NIH 3T3 fibroblasts and PCC7-S multipotent mouse cell lines (7) were next infected by G-MtkPL and G-MPL, respectively, and clones were isolated. Southern blot analysis of the DNA prepared from the clones demonstrated LTR-mediated duplication of I-*Sce*I *Phleo-lacZ* sequences (Fig. 4A). A *Bcl*I digestion generated the expected 5.8-kbp (G-MPL) or 7.2-kbp (G-MtkPL) fragments. The presence of two additional fragments corresponding to *Bcl*I sites in the flanking chromosomal DNA demonstrates a single proviral target in each isolated clone. The fact that the size of these *Bcl*I fragments varies from clone to clone indicates integration of retroviruses at distinct loci. I-*Sce*I digests show that I-*Sce*I recognition sites have been faithfully duplicated (5.8-kbp [G-MPL] or 7.2-kbp [G-MtkPL] fragments) (Fig. 4B).

I-*Sce*I-induced recombination leading to DNA exchange. The G-MtkPL virus confers a phleo^R β-galactosidase-positive (β-Gal$^+$) gls^S phenotype to NIH 3T3 cells, and G-MPL confers a phleo^R β-Gal$^+$ phenotype to PCC7-S cells (Fig. 2b). To allow

TABLE 1. Induced homologous recombination with I-*Sce*I[a]

Cell line or molecular event	No. of colonies							
	G418 + Gls[b]				G418			
	With I-*Sce*I[c]		Without I-*Sce*I[d]		With I-*Sce*I[c]		Without I-*Sce*I[d]	
	β-Gal$^+$	β-Gal$^-$	β-Gal$^+$	β-Gal$^-$	β-Gal$^+$	β-Gal$^-$	β-Gal$^+$	β-Gal$^-$
Cell lines								
NIH 3T3/G-MtkPL[e]								
Clone 1	0	66	0	0	69	581	93	0
Clone 2	0	120	0	0	15	742	30	0
PCC7-S/G-MPL[f]								
Clone 3					54	777	7	0
Clone 4					2	91	1	0
Clone 5					7	338	3	0
Molecular events[g]								
RI		0			8	0	6	
DsHR		15			0	19	0	
SsHR		0			0	4	0	
RI, Del		0			0	1	0	

[a] If an induced recombination between the provirus and pVRneo occurs, the cells acquire a neo^R β-Gal$^-$ phenotype. β-Gal expression phenotypes were determined by X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) histochemical staining.
[b] G418, Geneticin; Gls, ganciclovir. Ganciclovir has a general cytotoxicity, which explains the decrease in G418-resistant clones (23).
[c] Cotransfection with pVRneo and pCMV(I-*Sce*I+).
[d] Cotransfection with pVRneo and pCMV(I-*Sce*I−).
[e] One million cells of each clone were used.
[f] Five million cells of each clone were used.
[g] RI, random integration of pVRneo into the cell genome. DsHR, double-site HR; SsHR, single-site HR; RI, Del, random integration of pVRneo into the cell genome and simultaneous deletion of the provirus (see Fig. 2 and 5).

for direct selection of recombination events induced by I-*Sce*I, we constructed the pVRneo donor plasmid. In pVRneo, the *neo* gene is flanked by 300 bp homologous to sequences 5′ to the left chromosomal break and 2.5 kbp homologous to sequences 3′ to the right break (Fig. 2d). A polyadenylation signal was positioned 3′ to the *neo* gene to interrupt the *Phleo-lacZ* message following recombination. If an induced recombination between the provirus and the plasmid occurs, the resulting phenotype will be neo^R. Because of the presence of a polyadenylation signal in the donor plasmid, the *Phleo-lacZ* gene should not be expressed, resulting in a phleo^S β-Gal$^-$ phenotype. With G-MtkPL, it is possible to select simultaneously for the double break by negative selection with the *tk* gene (with ganciclovir) and for the integration of the donor plasmid with positive selection with the *neo* gene (with Geneticin). With G-MPL only the positive selection can be applied in medium containing Geneticin. Therefore, we expected to select both for HR and for integration of the donor plasmid near an active endogenous promoter. These two events can be distinguished, as an induced HR results in a neo^R β-Gal$^-$ phenotype and a random integration of the donor plasmid results in a neo^R β-Gal$^+$ phenotype.

Two different NIH 3T3/G-MtkPL and three different PCC7-S/G-MPL clones were then cotransfected with an expression vector for I-*Sce*I, pCMV(I-*Sce*I+), and the donor plasmid, pVRneo. Transient expression of I-*Sce*I may result in double-strand breaks at I-*Sce*I sites, therefore promoting HR with pVRneo. The control is the cotransfection with a plasmid which does not express I-*Sce*I, pCMV(I-*Sce*I−), and pVRneo.

NIH 3T3/G-MtkPL clones were selected either for loss of proviral sequences and acquisition of the neo^R phenotype (with ganciclovir and Geneticin) or for the neo^R phenotype only (Table 1). In the first case, 10^6 cells were transfected and 66 (clone 1) and 120 (clone 2) neo^R gls^R colonies were recovered, and no colonies were recovered in the control series. In addition, all neo^R gls^R colonies were β-Gal$^-$, an observation consistent with their resulting from HR at the proviral site. In the second case, 10^6 NIH 3T3/G-MtkPL cells were cotransfected with pVRneo and the pCMV(I-*Sce*I$^+$) plasmid and 650 (clone 1) and 757 (clone 2) neo^R colonies were recovered. In the control series, only 93 (clone 1) and 30 (clone 2) neo^R clones were recovered. Similar results were obtained with PCC7-S. In addition, 90% of the neo^R colonies were found to be β-Gal$^-$ [in the series with pCMV(I-*Sce*I+)]. This shows that expression of I-*Sce*I induces HR between pVRneo and the proviral site and that site-directed HR is 10 times more frequent than random integration of pVRneo near a cellular promoter and approximately 2 orders of magnitude more frequent than spontaneous HR. It should be noted that ganciclovir has a general cytotoxicity, which explains the decrease in neo^R β-Gal$^-$ clones in G418-ganciclovir selections (23).

Southern and Northern blot analyses of recombination events. The molecular structure of neo^R recombinants was examined by Southern blot (Fig. 5 and Table 1). If HR occurs at I-*Sce*I sites, we predict that digestion of recombinant DNA generates a 6.4-kbp *lacZ* fragment instead of the 4.2-kbp parental fragment. All 15 neo^R gls^R β-Gal$^-$ recombinants from NIH 3T3 cells exhibited only the 6.4-kbp *Kpn*I fragment. Therefore, the double-selection procedure leads only to the expected recombinants created by gene replacement (double-site homologous recombinants). The 25 β-Gal$^-$ recombinants generated from the single selection fell into four classes: (i) double-site homologous recombinants induced by I-*Sce*I as above (19 clones), (ii) integration of pVRneo in the left LTR as proven by the presence of a 4.2-kbp *Kpn*I fragment (corresponding to *Phleo-lacZ* in the remaining LTR), in addition to the 6.4-kbp fragment (Fig. 5 and Table 1) (single-site homologous recombinants; three independent β-Gal$^-$ recombinants from clone 1 and one β-Gal$^-$ recombinant from clone 3) (these clones correspond to I-*Sce*I HR in the left double-strand

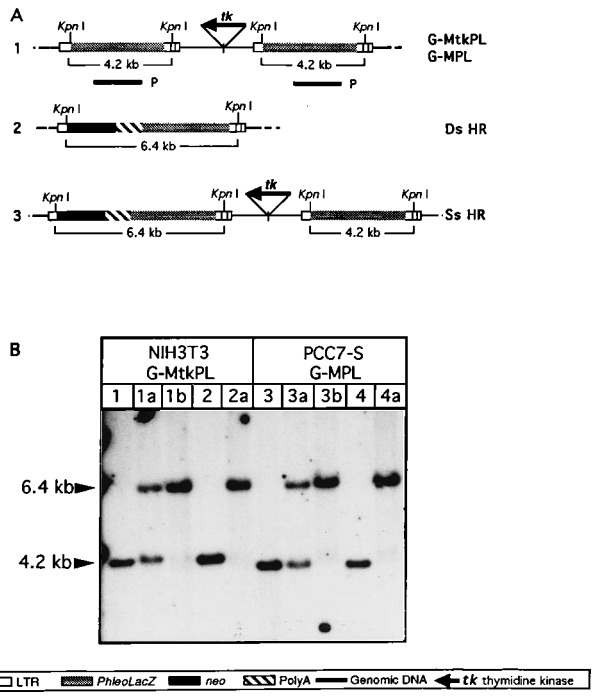

FIG. 5. Southern analysis of recombination. (A) Expected sizes of provirus fragments at the recombinant locus. 1, parental proviral locus. P, ^{32}P radioactively labelled probe used for hybridization. 2, a recombinant derived after cleavage at the two I-*Sce*I sites followed by double-break repair using pVRneo (double-site homologous recombination [Ds HR]). 3, a recombination event initiated by the cleavage at the I-*Sce*I sites in the left LTR (single-site homologous recombination [Ss HR]). (B) Southern analysis of DNA from NIH 3T3/G-MtkPL clones 1 and 2, PCC7-S/G-MPL clones 3 and 4, and transformants derived from cotransfection with pCMV(I-*Sce*I+) and pVRneo (1a, 1b, 2a, 3a, 3b, and 4a). *Kpn*I digestion of the parental DNA generates a 4.2-kbp fragment containing *lacZ* sequences. *Kpn*I digestion of the recombinants which are repaired generates a 6.4-kbp fragment. Recombinants 1a and 3a are examples of Ds HR. Recombinants 1b, 2a, 3b, and 4a are examples of Ss HR.

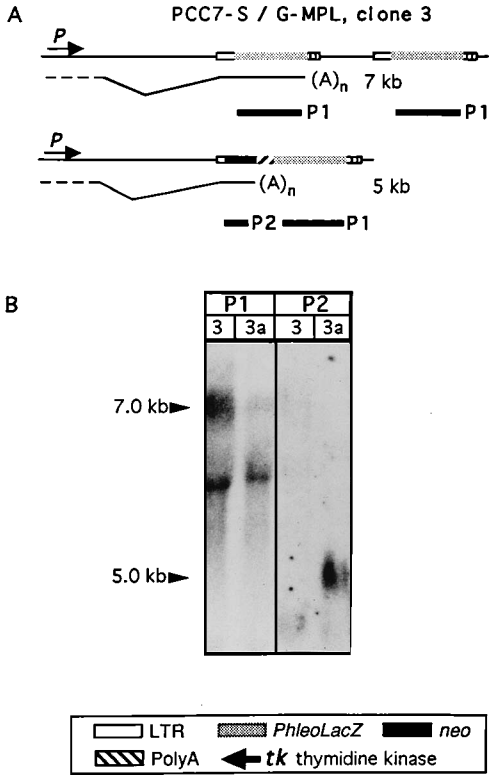

FIG. 6. Northern analysis of recombination. (A) Expected structures and sizes of RNAs from PCC7-S/G-MPL clone 3 cells before (upper diagram) and after (lower diagram) I-*Sce*I induced HR with pVRneo. P1 and P2, ^{32}P radioactively labelled probes. (B) Northern blot analysis of the PCC7-S/G-MPL clone 3 recombinant (total RNA). Lanes 3, parental cells; lanes 3a, recombinant cells. Lanes P1, probe *LacZ*; lanes P2, probe *neo*. Parental PCC7-S/G-MPL clone 3 cells express a 7.0-kb *lacZ* RNA, as expected for the trapping of a cellular promoter leading to expression of a cell-virus fusion RNA. The recombinant clone does not express this *lacZ* RNA but expresses a *neo* RNA of 5.0 kb, corresponding to the size expected for an accurate replacement of *Phleo-lacZ* by the *neo* gene.

break only or [less likely] to a double crossover between LTR and pVRneo), (iii) random pVRneo integrations into the cell genome (Table 1), and (iv) random pVRneo integration and simultaneous deletion of provirus (one β-Gal$^-$ recombinant). We suggest that the fourth class may correspond to repair of double-strand breaks with the homologous chromosome. As expected, all β-Gal$^+$ recombinants from Geneticin selection alone correspond to random pVRneo integrations, whether they originated from the experimental series (eight clones analyzed) or from the control series (six clones analyzed).

We obtained additional evidence that recombination had occurred at the I-*Sce*I site of PCC7-S/G-MPL 1 by analyzing the RNAs produced in parental and in recombinant cells (Fig. 6). Parental PCC7-S/G-MPL 1 cells express a 7.0-kb *lacZ* RNA indicative of trapping of a cellular promoter leading to expression of a cell-virus fusion RNA. The recombinant clone does not express this *lacZ* RNA but expresses a *neo* RNA of 5.0 kb. The size of the *neo* RNA corresponds to the exact size expected for an accurate exchange of *Phleo-lacZ* by the *neo* gene and uses of the same cellular and viral splice sites (viral *Phleo-lacZ* RNA in the LTR is 3.7 kb, and *neo* RNA in pVRneo is 1.7 kb).

DISCUSSION

The results presented here demonstrate that double-strand breaks can be induced by the I-*Sce*I system of *S. cerevisiae* in mammalian cells and that the breaks in the target chromosomal sequence induce site-specific recombination with input plasmid donor DNA.

To operate in mammalian cells, the system requires endogenous I-*Sce*I-like activity to be absent from mammalian cells and I-*Sce*I protein to be neutral for mammalian cells. It is unlikely that endogenous I-*Sce*I-like activity operates in mammalian cells, as the introduction of I-*Sce*I recognition sites does not appear to lead to rearrangement or mutation of the DNA sequences. For instance, all NIH 3T3 and PCC7-S clones infected with a retrovirus containing the I-*Sce*I restriction site propagated the virus stably. To test for the toxicity of the I-*Sce*I gene product, an I-*Sce*I-expressing plasmid was introduced into the NIH 3T3 cell line (data not shown). A very high percentage of cotransfer of a functional I-*Sce*I gene was found, suggesting no selection against this gene. Functionality of the I-*Sce*I gene was demonstrated by analysis of transcription, by immunofluorescence detection of the gene product and biological function (5a).

We next tested whether the endonuclease would cleave a recognition site placed on a chromosome. This was accomplished by placing two I-*Sce*I recognition sites separated by 5.8 or 7.2 kbp on a chromosome in each LTR of proviral structures and by analyzing the products of a recombination reaction with a targeting vector in the presence of the I-*Sce*I protein. Our

results indicate that, in the presence of I-*Sce*I, the donor vector recombines very efficiently with sequences within the two LTRs to produce a functional *neo* gene. This suggests that I-*Sce*I very efficiently induced double-strand breaks in both I-*Sce*I sites. In addition, as double-strand breaks were obtained with five distinct proviral insertions, the ability of the I-*Sce*I protein to digest an I-*Sce*I recognition site is not highly dependent on surrounding structures. The demonstration of the ability of the I-*Sce*I enzyme to have biological function on chromosomal sites in mammalian cells paves the way for a number of manipulations of the genome in living organisms. In comparison with site-specific recombinases (9, 18), the I-*Sce*I system is nonreversible. Site-specific recombinases locate not only the sites for cutting the DNA but also the sites for rejoining by bringing together the two partners. In contrast, the only requirement with the I-*Sce*I system is homology of the donor molecule with the region flanking the break induced by I-*Sce*I protein. The limitation of the I-*Sce*I system is that the I-*Sce*I site must first be targeted to the chromosome.

Our results indicate for the first time that double-strand DNA breaks in chromosomal targets stimulate HR with exogenous DNA in mammalian cells. Because we used a combination of double-strand breaks in chromosomal recipient DNA and super-coiled donor DNA, we suspected that the stimulation of recombination by the I-*Sce*I endonuclease is the double-strand break repair mechanism (21). Therefore, the induced break is probably repaired by a gene conversion event involving the concerted participation of both broken ends which invade and copy DNA from the donor copy after creation of a single-stranded region by 5′-to-3′ exonucleolytic digestion. However, a number of studies of recombination in mammalian cells and in *S. cerevisiae* (10, 11, 19) suggest that there is an alternative pathway of recombination, termed single-strand annealing. In the single-strand annealing mechanism double-strand breaks are substrates in the action of an exonuclease that exposes homologous complementary single-strand DNA on the recipient and donor DNAs. Annealing of the complementary strand is then followed by a repair process that generates recombinants. The I-*Sce*I system can be used to evaluate the relative importance of the two mechanisms.

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