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Spontaneous and restriction enzyme-induced chromosomal recombination in mammalian cells

(intrachromosomal recombination/interchromosomal recombination/double-strand break)

ALAN R. GODWIN†, RONI J. BOLLAG‡, DONNA-MARIE CHRISTIE, AND R. MICHAEL LISKAY§

Departments of Genetics and Therapeutic Radiology, Yale University School of Medicine, New Haven, CT 06510

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ABSTRACT **We have derived Chinese hamster ovary (CHO) cell hybrids containing herpes simplex virus thymidine kinase (tk) heteroalleles for the study of spontaneous and restriction enzyme-induced interchromosomal recombination. These lines allowed us to make a direct comparison between spontaneous intrachromosomal and interchromosomal recombination using the same *tk* heteroalleles at the same genomic insertion site. We find that the frequency of interchromosomal recombination is less by a factor of at least 5000 than that of intrachromosomal recombination. Our results with mammalian cells differ markedly from results with *Saccharomyces cerevisiae*, with which similar studies typically give only a 10- to 30-fold difference. Next, to inquire into the fate of double-strand breaks at either of the two different *Xho* I linker insertion mutations, we electroporated *Pae*R7I enzyme, an isoschizomer of *Xho* I, into these hybrids. *A priori*, these breaks can be repaired either by recombination from the homolog or by end-joining. Despite a predicted bias against recovering end-joining products in our system, all cells characterized by enzyme-induced resistance to hypoxanthine/aminopterin/thymidine were, in fact, due to nonhomologous recombination or end-joining. These results are in agreement with other studies that used extrachromosomal sequences to examine the relative efficiencies of end-joining and homologous recombination in mammalian cells, but are in sharp contrast to results of analogous studies in *S. cerevisiae*, wherein only products of homologous events are detected.**

Studies using introduced, extrachromosomal DNA substrates have shown that both end-joining (nonhomologous recombination) and homologous recombination occur in mammalian somatic cells; however, the process of end-joining occurs far more frequently than homologous recombination (1). When linear simian virus 40 (SV40) genomes containing terminal homology were examined for the relative rates of nonhomologous end-joining and homologous recombination in mammalian cells, end-joining was about 3-fold more efficient than homologous recombination (2). Similarly, repair of transfected plasmids occurs primarily by end-joining in mammalian cells (1). Restriction enzyme-induced breaks in mammalian episomal vectors are also repaired by end-joining or by religation of cohesive ends (3). Random integration generally prevails over gene-targeting (4), again suggesting a bias against homologous recombination in mammalian cells.

In this report, we present a study of the relative efficiencies of end-joining and homologous recombination at a defined *in vivo* double-strand break in a mammalian chromosome. Since differences exist between extrachromosomal recombination and intrachromosomal recombination (5) as well as between gene-targeting and intrachromosomal recombination (6) in mammalian cells, we sought to determine whether the fate of chromosomal breaks differed from the fate of extrachromosomal breaks.

To examine directly the relative efficiencies of end-joining and homologous recombination at directed double-strand breaks, we have generated thymidine kinase (tk)-lacking Chinese hamster ovary (CHO) cell hybrids carrying herpes simplex virus (HSV) *tk* heteroalleles. These lines carry two different *Xho* I linker insertion mutations allowing directed breaks to be created *in vivo* by electroporating the appropriate restriction endonuclease. We used *Pae*R7I, since its isoschizomer, *Xho* I, was not active under the electroporation conditions used. These lines were used to examine both spontaneous and restriction enzyme-induced interchromosomal recombination.

Because the interchromosomal recombination substrates generated in this study are derived from one particular intrachromosomal recombination substrate, we were also able to compare intragenic intrachromosomal and interchromosomal recombination frequencies using the same heteroalleles at the same genomic site.

MATERIALS AND METHODS

Cell Culture. CHO cells were cultured as described for mouse L cells (6). At each step Southern blot analysis was used to identify appropriate lines and to verify their genotype. Ploidy was determined at each step by karyotype analysis as described (7).

Hypoxanthine/Aminopterin/Thymidine (HAT) Selection. Cells were plated at a density of 3×10^6 cells per 150-cm^2 flask in HAT medium (8). Cells were refed after 4 days, and surviving colonies were counted after 14 days.

Trifluorothymidine Selection. Cells were plated at densities of $1–4 \times 10^5$ in 100-mm^2 dishes containing Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (vol/vol) fetal calf serum (FBS) and 5 μg of trifluorothymidine (Sigma) per ml, and selection was continued for 2 weeks.

Generation of Resistant Cells. Ten micrograms of pSV2gpt (9) in which gpt is the gene coding for guanine/hypoxanthine phosphoribosyltransferase [or pSV2*hyg in which hyg is the gene coding for hygromycin B resistance (Hyg^R)] plasmid and 1 μl 1 M 2-mercaptoethanol were added to 5×10^6 cells in 0.5 ml of Hepes-buffered 0.15 M NaCl. Electroporation was carried out with a Promega model 450 electroporator at a capacitance of 250 μF, 400 V, and an electrode gap of 1 cm for 150 msec. Surviving cells were plated at densities of 1 ×

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Abbreviations: HAT, hypoxanthine/aminopterin/thymidine; tk/TK, thymidine kinase; HSV, herpes simplex virus; SV40, simian virus 40; gpt, guanine/hypoxanthine phosphoribosyltransferase; Hyg, hygromycin B; superscript R, resistant/resistance.

†Present address: Department of Human Genetics, University of Utah, Salt Lake City, UT 84112.
‡Present address: Institute for Molecular Medicine and Genetics, Medical College of Georgia, Augusta, GA 30912.
§To whom reprint requests should be sent at the present address: Department of Molecular and Medical Genetics, L103, Oregon Health Sciences University, Portland, OR 97201.

10^4 or 1×10^5 per 100-mm^2 dish in DMEM supplemented with 10% FBS and allowed to recover for 2 days. Cells were cultured subsequently in MAAX medium [DMEM supplemented with 10% FBS, 60 μM mycophenolic acid (Eli Lilly), 11.5 μM adenine (Sigma), 0.98 μM azaserine (Sigma), and 2 mM xanthine] or in medium containing 800 μg of hygromycin B (Hyg; Calbiochem) per ml, and selection proceeded in each case for 2 weeks.

Generation of Hybrid Cell Lines. Hyg^R and MAAX^R CHO cell lines were each plated at a density of 3×10^5 per 60-mm^2 dish and fused the next day by using PEG-1000 (Sigma) as described (10). The following day cells were plated at densities of 1×10^4 or 1×10^5 per 100-mm dish containing MAAX medium supplemented with 600 μg of hygromycin per ml, and selection proceeded for 2 weeks. One line recovered carries solely a triplication of *tk8*. This line is presumed to result from fusion of a Hyg^R *tk8* cell that had undergone unequal sister chromatid exchange to generate a triplication of *tk8*, with a MAAX^R cell that had lost the chromosome carrying the duplication of *tk26*.

Molecular Analysis of Recombinants. Techniques used for the preparation of genomic DNA and Southern blot analysis have been described (6).

***Pae*R7I Electroporation.** *Pae*R7I (180–300 units; 60 units/μl; New England Biolabs) was electroporated into 10^7 cells by using a Bio-Rad Gene Pulser as described (7). Cells were plated in pools of 10^6 cells in nonselective medium and allowed to recover for 2 days before culture in HAT medium. Cells were refed with HAT medium after 4 days in selection.

Plasmid Rescue and DNA Sequencing. DNA was rescued as described (11) after cleavage with *Bam*HI or *Hin*dIII, and both the *Xho* I-resistant allele and *Xho* I-sensitive allele were sequenced by using a United States Biochemical Sequenase II kit.

RESULTS

Generation of Experimental Lines. We have generated substrates to compare intrachromosomal recombination, where the recombining *tk* alleles are closely linked on one chromosome, with interchromosomal recombination, where the *tk* alleles are present on homologous chromosomes. Intrachromosomal recombination can occur by intrachromatid or sister chromatid interactions, whereas interchromosomal recombination requires interaction between homologs (5). For both types of recombination, both crossing over and gene conversion can generate productive tk$^+$ recombinants.

The protocol for generating the recombination substrates is shown in Fig. 1. The starting line (R.J.B. and R.M.L., unpublished data), a derivative of CHO AT3-2 2E-5 cells (12), carries an intrachromosomal recombination substrate, pJS3 (13), containing two closely linked *tk* genes carrying different *Xho* I linker insertion mutations that flank the *neo* gene (see Fig. 1). This parental line was cultured initially in HAT medium containing G418 (which selects for the *neo* gene) to select *tk*$^+$ gene convertants—i.e., recombinants that retained a *tk* duplication. Southern blot analysis (data not shown) was used to determine which *tk* allele had been converted to wild type (see Fig. 1, step A). Trifluorothymidine selection (Fig. 1, step B) was used subsequently to select against the wild type *tk* gene. We isolated both gene conversion products, which now carry the same *Xho* I linker insertion in both *tk* genes (Fig. 1, step B1), and crossover products (Fig. 1, step B2), which carry a single mutant *tk* gene.

Southern blot analysis confirmed generation of the "8 neo 8" and "26 neo 26" lines as defined in Fig. 1 and is shown in Fig. 2*A*. *Hin*dIII digestion generated the same 13- and 2.0-kb fragments for both the 8 neo 8 (lane 1) and 26 neo 26 (lane 3) lines. In contrast, diagnostic fragments resulted from *Hin*dIII/*Xho* I digestion: 5.4- and 5.0-kb bands as well as a 1.0-kb

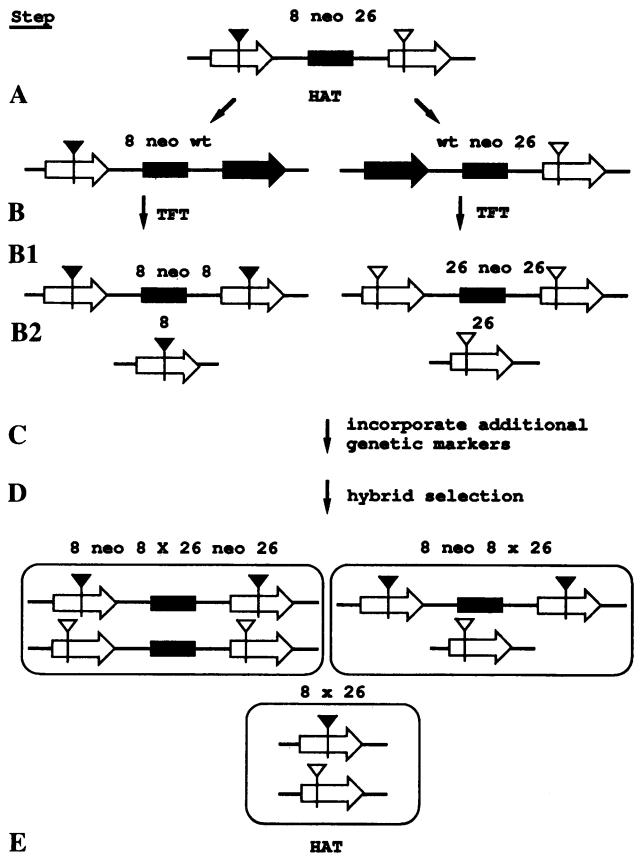

FIG. 1. Flow chart of derivation of experimental lines. Open arrows represent mutant *tk* genes, black arrows represent wild-type *tk* genes, black rectangles denote the *neo* gene, 8 denotes the *tk8* allele, 26 denotes the *tk26* allele, the black triangle denotes the *tk8* allele *Xho* I linker insertion mutation, and the open triangle denotes the *tk26* allele *Xho* I linker insertion mutation. TFT, trifluorothymidine; *neo*, gene conferring resistance to neomycin.

doublet for the 8 neo 8 line (lane 2) and 5.9-, 4.5-, 1.5-, and 0.5-kb bands for the 26 neo 26 line (lane 4). The 0.5-kb band is not detectable under the electrophoresis conditions shown in Fig. 2.

Lines carrying one or two *tk8* alleles were transformed by electroporation with pSV2*hyg. Similarly, lines carrying one or two *tk26* alleles were transformed with pSV2gpt (ref. 9; Fig. 1, step C). Subsequently, polyethylene glycol-mediated cell fusion generated Hyg^R, Gpt^R tetraploid CHO cell lines that carry one or two *tk26* alleles on one chromosome and one or two *tk8* alleles on the homologous chromosome (Fig. 1, step D).

Southern blot analysis of one "8 neo 8 × 26 neo 26" line is shown in Fig. 2. *Hin*dIII digestion (lane 5) generates only the 13- and 2.0-kb fragments diagnostic of either the 8 neo 8 line (lane 1) or the 26 neo 26 line (lane 3). *Hin*dIII/*Xho* I digestion (lane 6) generates the 5.4- and 5.0-kb fragments as well as a 1.0-kb doublet from the 8 neo 8 chromosome (lane 2) and the 5.9-, 4.5-, 1.5-, and 0.5-kb fragments from the 26 neo 26 chromosome (lane 4). Therefore, this line contains the expected *tk* substrate configuration. The *tk* substrate configuration of other lines used in this study was confirmed similarly.

Spontaneous Recombination Frequencies. The spontaneous reversion frequency was found to be 5.5×10^{-7} with a diploid line carrying only a single copy of *tk26*, while a tetraploid line carrying a triplication of *tk8* (see *Materials and Methods*) reverted at a frequency of 4.9×10^{-8}. Although the *tk* genes in these HAT^R cells have not been sequenced, the cells are presumed to result from either reversion of the endogenous

CHO *TK* locus or from frameshift mutations that restore reading frame in the HSV *tk* gene.

An example of a spontaneous revertant is shown in Fig. 2*A*, lanes 8 and 9. This revertant carries the same *Hin*dIII and *Hin*dIII/*Xho* I bands as found with the parent line 8 neo 8 × 26 neo 26 (lanes 6 and 7). A true recombinant should have generated either a 2.0-kb or 13-kb *Hin*dIII fragment that is resistant to *Xho* I cleavage.

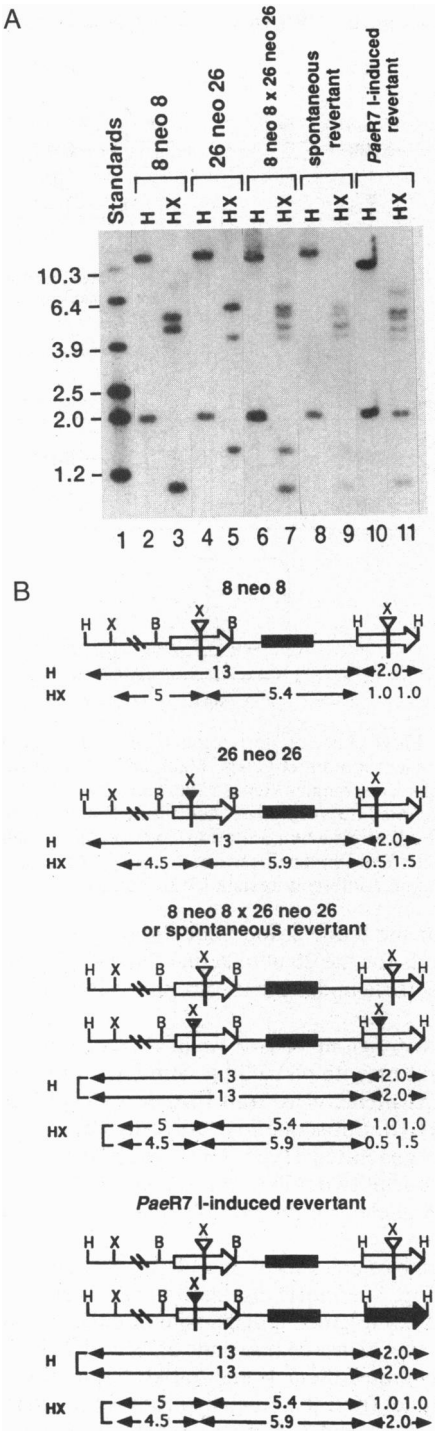

FIG. 2. (*A*) Southern blot of experimental lines and revertants as designated in Fig. 1. The additional band ≈7.5–8.5 kb long in lanes 3, 7, and 11 most likely results from partial digestion. Lanes: H, *Hin*dIII digestion; HX, *Hin*dIII/*Xho* I digestion. (*B*) Origin of bands seen in Southern blot. Symbols are as described for Fig. 1. The solid black arrow denotes an *Xho* I-resistant *tk* gene. *Hin*dIII digestion of the *Pae*R7I-induced HAT^R line (lane 9) generates the same fragments as the interchromosomal substrate parent (lane 5). *Hin*dIII/*Xho* I digestion, again, generates the bands expected for the interchromosomal parent except that the 1.5- and 0.5-kb bands have been replaced by an 2.0-kb band resistant to *Xho* I cleavage.

Three different types of interchromosomal recombination lines were generated: lines with a single copy of each mutant *tk* allele (8 × 26), lines carrying a duplication of each of the *tk* alleles (8 neo 8 × 26 neo 26), and lines carrying a duplication of *tk8* and a single copy of *tk26* (8 neo 8 × 26). The spontaneous recombination frequencies were compared with a line carrying closely linked copies of the *tk8* and *tk26* alleles (8 neo 26) at the same genomic site. Although occasional HAT^R colonies were obtained when using the interchromosomal recombination substrates, the frequency was never higher than the spontaneous reversion frequency. Southern blot analysis of these colonies never revealed an authentic interchromosomal recombinant (i.e., a *Xho* I-resistant gene). The spontaneous frequency of interchromosomal recombination was $<3.6 \times 10^{-9}$ when the data for all three types of interchromosomal recombination lines were combined, whereas the intrachromosomal recombination frequency was 6.7×10^{-5} (see Table 1). Six intrachromosomal recombination lines (8 neo 26) carrying the same plasmid integrated at different genomic sites showed at a maximum only a 20-fold variation in recombination frequency (5). Therefore, the difference between intrachromosomal and interchromosomal recombination is far greater than can be explained by variation between experiments or CHO cell lines.

It was possible that the tetraploid nature of the interchromosomal recombination substrate affected recombination. Therefore, we generated a tetraploid intrachromosomal recombination substrate by fusing a MAAX^R (*gpt*$^+$) derivative of the original intrachromosomal parent line 8 neo 26 (Fig. 1) to a Hyg^R derivative of the intrachromosomal parent line 8 neo 26. As shown in Table 1, we observed no difference in the intrachromosomal recombination frequency between the diploid and tetraploid cells. Thus, there is a 4-order-of-magnitude drop in mammalian interchromosomal recombi-

Table 1. Spontaneous recombination frequencies

Substrate cell line	Ploidy	Recombination frequency*	
		Cell subline	Events per no. of cells plated
Intrachromosomal			
8 neo 26	2N		6.7×10^{-5}
8 neo 26 × 8 neo 26	4N		5.0×10^{-5}
Interchromosomal			
8 × 26	4N	1	$<2.6 \times 10^{-8}$
		2	$<2.7 \times 10^{-8}$
		3	$<2.0 \times 10^{-8}$
		4	$<3.5 \times 10^{-8}$
		Total†	$<9.3 \times 10^{-9}$
8 neo 8 × 26	4N		$<3.8 \times 10^{-8}$
8 neo 8 × 26 neo 26	4N	1	$<2.4 \times 10^{-8}$
		2	$<4.3 \times 10^{-8}$
		3	$<3.3 \times 10^{-8}$
		Total†	$<1.0 \times 10^{-8}$
All		Total‡	$<3.6 \times 10^{-9}$

*Although spontaneous HAT^R revertants were detected in these experiments, Southern blot analysis revealed that the *tk* genes were sensitive to cleavage by *Xho* I. Since HAT^R *tk* genes sensitive to *Xho* I cleavage cannot be generated by recombination, these numbers are not included here.

†The total recombination frequency was determined by assuming 1 recombination event had occurred for the total number of cells plated for that substrate.

‡The total recombination frequency was determined by assuming 1 recombination event had occurred for the total number of cells plated for all interchromosomal substrates.

nation compared with intrachromosomal recombination for sequences at the same genomic site.

Restriction Enzyme-Induced Recombination. We were next interested in generating double-strand breaks in our interchromosomal recombination substrate to assess whether the breaks would be repaired preferentially by recombination with the *tk* alleles on the homologous chromosome or by end-joining to yield HAT^R cells. We generated the double-strand breaks by electroporating *Pae*R7I into an interchromosomal recombination substrate carrying duplications of the *tk8* and *tk26* linker insertion alleles (8 neo 8 × 26 neo 26 line). We chose this substrate, since the additional *tk* genes should provide additional sites for *Pae*R7I cleavage and allow a more sensitive assay than the 8 × 26 line. Electroporation of 180, 240, or 360 units of enzyme generated HAT^R cells at a frequency of 1×10^{-6}, compared with the spontaneous reversion frequency of 2.4×10^{-8}. Electroporation of *Pae*R7I storage buffer generated no induction of HAT^R cells. Finally, electroporation of *Pae*R7I into a tetraploid line containing a tandem triplication of *tk8* did not induce HAT^R cells (data not shown).

Examination of HAT^R cell lines by Southern blot analysis revealed that 58% (7 of 12 independent lines) now contained a *Xho* I-resistant *tk* gene (data not shown). All of the *Xho* I-resistant lines have alterations in a *tk26* allele. Four of the genes resistant to *Xho* I cleavage were *tk26* genes flanked by *Bam*HI cleavage sites and three were *tk26* genes flanked by *Hin*dIII sites, demonstrating there was no bias for recovery of alterations between the *tk26* genes. Southern blot analysis of a representative *Pae*R7I-induced HAT^R line shown in Fig. 2 indicates that the *tk26* allele flanked by *Hin*dIII sites has become resistant to *Xho* I cleavage. Therefore, either homologous recombination has corrected the linker insertion mutation or a nonhomologous recombination event has removed the *Xho* I site and restored the *tk* gene reading frame.

To determine the basis for resistance to *Xho* I digestion, all seven independent *Xho* I-resistant genes were isolated by plasmid rescue and sequenced in the vicinity of the *Xho* I linker insertion site. As predicted from the Southern blot analysis, all alterations occurred in a *tk26* allele and restored the *tk* reading frame, but none regenerated a truly wild type sequence (see Fig. 3). For example, revertant 1 is consistent with cleavage by *Pae*R7I followed by fill-in of both ends and blunt-end ligation. Revertants 2–5 are consistent with cleavage, resection to various degrees, and ligation. Finally, revertant 7 was generated by cleavage, resection, and insertion of an unidentified 33-bp DNA fragment. Thus, all seven revertants have corrected the *tk26* frameshift and show deletion or addition of amino acids relative to the wild-type gene. In each case, there was no alteration of the *tk8* allele that was rescued from the homologous chromosome (data not shown). Therefore, these seven genes resistant to *Xho* I cleavage likely resulted from nonhomologous end-joining. The *tk* genes from the five HAT^R lines with genes sensitive to *Xho* I cleavage have not been sequenced, since the entire coding region from all four genes would have to be scanned for mutations; however, since several other mutagens induced HAT^R lines sensitive to *Xho* I cleavage without inducing recombination, possibly these lines result from activation of mutagenesis due to the presence of double-strand breaks.

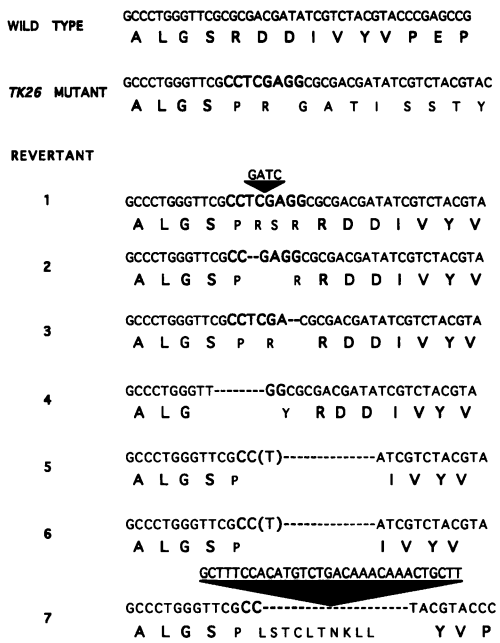

FIG. 3. Sequence of *Pae*R7I-induced *tk* revertants. Hyphens denote deleted bases, black inverted triangles denote insertions, amino acids in boldface letters and larger font depict amino acids corresponding to the tk protein, bases in a larger font represent bases derived from the *Xho* I linker insertion mutation, and bases in parentheses denote bases whose origin is ambiguous (i.e., the T could remain from either side of the deletion).

DISCUSSION

We constructed CHO hybrid cell lines carrying HSV *tk* heteroalleles to examine spontaneous and restriction enzyme-induced interchromosomal recombination. We demonstrated that (*i*) the frequency of spontaneous intrachromosomal recombination greatly exceeds the frequency of interchromosomal recombination at the same genomic site in mammalian cells and (*ii*) that repair of double-strand breaks at a defined chromosomal site in mammalian cells occurs by end-joining more frequently than by homologous recombination.

Three different types of experimental lines were generated: (*i*) lines carrying a single copy of each mutant *tk* allele, (*ii*) lines carrying a linked duplication of each of the *tk* alleles, and (*iii*) lines carrying a linked duplication of *tk8* and a single copy of *tk26* (see Fig. 1). The spontaneous recombination frequency in each of these lines was compared to that in a line carrying closely linked copies of the *tk8* and *tk26* alleles at the same genomic site. Although we did not recover any true spontaneous interchromosomal recombinants, given the number of cells we plated, our results are consistent with the low spontaneous interchromosomal recombination frequency at the human *TK* locus (14, 15). We determined that the spontaneous frequency of interchromosomal recombination was $<3.6 \times 10^{-9}$, while the intrachromosomal recombination frequency at the identical genomic site was 6.7×10^{-5}. This large decrease in recombination frequency could not be explained by the tetraploid nature of the interchromosomal recombination substrates because tetraploid and diploid intrachromosomal recombination substrates had identical recombination frequencies. Thus, at the CHO cell genomic site that we studied, interchromosomal recombination is at least 3 orders of magnitude less frequent than intrachromosomal recombination.

In contrast, only a 6- to 30-fold difference in frequency between intra- and interchromosomal recombination has been documented in *Saccharomyces cerevisiae*. In one study, when *his4* heteroalleles were examined, interchromosomal recombination was observed to be less frequent by a factor of 30 than intrachromosomal recombination (16). A more comprehensive study compared interchromosomal recombination at five different genomic locations with intrachromosomal recombination (17). Allelic recombination was less frequent by a factor of 6–10 than intrachromosomal recombination at the various sites.

Two issues may relate to the contrasting *S. cerevisiae* and mammalian findings. First, there may be a "dilution effect" in mammals because the mammalian genome is about 200-fold larger than that of yeast and because the mammalian

nuclear volume is ≈25-fold larger than a diploid yeast nucleus. Alternatively, interchromosomal recombination may be "repressed" in mammalian cells because translocations and other deleterious interactions might result from recombination between numerous, highly repeated sequences, such as *Alu* I repeats.

We electroporated *Pae*R7I into a line carrying an interchromosomal recombination substrate consisting of duplications of *tk26* and *tk8* alleles (8 neo 8 × 26 neo 26 line) to compare the relative efficiencies of induced end-joining and homologous recombination. Although the frequency of HAT^R cells increased, none of the recovered products are consistent with homologous recombination. All seven revertants resistant to *Xho* I cleavage resulted from end-joining. Therefore, the restriction enzyme-induced frequency of interchromosomal recombination was still below our detection threshold. End-joining clearly predominated over homologous recombination in our study. This bias has been demonstrated previously when using extrachromosomal substrates for both primary (18) and established cell lines (2, 19), but here we describe a chromosomal demonstration of this bias.

It should be noted that the bias toward end-joining is likely to be even greater than we observe. Recovery of resection and end-joining products is constrained in our system, since only those events that restore reading frame and generate permissible amino acid substitutions, insertions, and deletions will produce HAT^R cells. Furthermore, any events in which the complementary ends produced by *Pae*R7I digestion are ligated without any resection will regenerate the original *Xho* I linker insertion mutation. In contrast, homologous recombination should always generate functional tk enzyme. In addition, we never recovered alterations of a *tk8* allele, again suggesting that the products we recovered underestimate the amount of *Pae*R7I cleavage in our recombination substrate. Cleavage at the *tk8* site might be constrained by the *tk* sequence surrounding the linker insertion. Alternatively, alterations of amino acid sequence that retain tk enzymatic activity may be more limiting at the *tk8* linker insertion site than at the *tk26* site. Despite the bias of our system against end-joining, we only recovered end-joining events.

Our cell lines require recombinational repair to occur from the homologous chromosome to generate a recoverable HAT^R cell line, since sister chromatid recombination simply regenerates the original *Xho* I linker insertion mutation alleles. Although homolog recombination is used to repair double-strand breaks in both yeast (20, 21) and *Drosophila* (22, 23), sister chromatid recombination predominates. An earlier study from our lab showed that the majority of mammalian intrachromosomal crossing over is due to sister chromatid interactions, suggesting that mammalian cells have a bias toward sister chromatid recombination (24). A bias toward sister chromatid recombination could explain in part our inability to obtain homologous recombinants after generation of directed breaks.

The end-joining products we observe are consistent with the resection and ligation products observed at breaks in transfected SV40 genomes (1), at breaks generated *in vivo* in an extrachromosomally replicating plasmid by electroporation of restriction enzymes (3), and at breaks processed by *Xenopus* egg (25) and oocyte extracts (26). Therefore, our results extend these studies to double-strand breaks generated within mammalian chromosomal DNA. All but one of our revertants could arise after *Pae*R7I cleavage, subsequent resection or fill-in, and ligation. One intriguing revertant harbors an insertion of an unidentified fragment of DNA into a resected *Pae*R7I site to restore *tk* reading frame. This type of product was first detected by using transfected SV40 genomes (27). Five similar insertions of random genomic DNA were also recovered at *in vivo* breaks in an extrachromosomal plasmid following *Pvu* II electroporation (3). Recovery of a product of such an event that only rarely should restore the *tk* reading frame and form a functional protein again underscores the predominance of end-joining over homologous recombination. Finally, revertants 5 and 6 have a 1-bp ambiguity regarding the location of the junction (Fig. 3). This type of microhomology is seen in many end-joining systems (1) and is believed to result from complementary base pairing of short homologies during end-joining.

In conclusion, we used CHO cell hybrids to document at least a 3-order-of-magnitude difference in the frequencies of mammalian intra- and interchromosomal recombination. In addition, we found that end-joining predominated over homologous recombination at directed chromosomal breaks in mammalian cells.

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1. Roth, D. & Wilson, J. (1989) in *Genetic Recombination*, eds. Kucherlapati, R. & Smith, G. R. (Am. Soc. Microbiol., Washington, DC), pp. 621–653.
2. Roth, D. B. & Wilson, J. H. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 3355–3359.
3. Winegar, R. A., Lutze, L. H., Rufer, J. T. & Morgan, W. F. (1989) *Mutagenesis* **7,** 439–445.
4. Thomas, K. R., Folger, K. R. & Capecchi, M. R. (1986) *Cell* **44,** 419–428.
5. Bollag, R. J., Waldman, A. S. & Liskay, R. M. (1989) *Annu. Rev. Genet.* **23,** 199–225.
6. Godwin, A. R. & Liskay, R. M. (1994) *Genetics* **136,** 607–617.
7. Ager, D. D., Phillips, J. W., Coulmna, E. A., Winegar, R. A. & Morgan, W. F. (1991) *Radiat. Res.* **128,** 150–156.
8. Szybalski, W., Szybalska, E. H. & Ragni, G. (1962) *Natl. Cancer Inst. Monogr.* **7,** 75–88.
9. Mulligan, R. C. & Berg, P. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 2072–2076.
10. Davidson, R. L., O'Malley, K. A. & Wheeler, T. B. (1976) *Somatic Cell Genet.* **2,** 271–280.
11. Folger, K. R., Thomas, K. & Capecchi, M. R. (1985) *Mol. Cell. Biol.* **5,** 59–69.
12. Nairn, R. S., Adair, G. M. & Humphrey, R. M. (1982) *Mol. Gen. Genet.* **187,** 384–390.
13. Liskay, R. M., Stachelek, J. L. & Letsou, A. (1984) *Cold Spring Harbor Symp. Quant. Biol.* **69,** 183–188.
14. Benjamin, M. B., Potter, H., Yandell, D. W. & Little, J. B. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 6652–6656.
15. Benjamin, M. B. & Little, J. B. (1992) *Mol. Cell. Biol.* **12,** 2730–2738.
16. Jackson, J. A. & Fink, G. R. (1981) *Nature (London)* **292,** 306–311.
17. Lichten, M. & Haber, J. E. (1989) *Genetics* **123,** 261–268.
18. Zheng, H., Chang, X.-B. & Wilson, J. H. (1989) *Plasmid* **22,** 99–105.
19. Chang, X.-B. & Wilson, J. H. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 4959–4963.
20. Kadyk, L. C. & Hartwell, L. H. (1992) *Genetics* **132,** 387–402.
21. Kadyk, L. C. & Hartwell, L. H. (1993) *Genetics* **133,** 469–487.
22. Engels, W. R., Johnson-Schiltz, D. M., Eggleston, W. B. & Sved, J. (1990) *Cell* **62,** 515–525.
23. Johnson-Schiltz, D. M. & Engels, W. R. (1993) *Mol. Cell. Biol.* **13,** 706–7018.
24. Bollag, R. J. & Liskay, R. M. (1991) *Mol. Cell. Biol.* **11,** 4839–4845.
25. Pfeiffer, P. & Vielmetter, W. (1988) *Nucleic Acids Res.* **16,** 907–924.
26. Lehman, C. W., Trautman, J. K. & Carroll, D. (1994) *Nucleic Acids Res.* **22,** 434–442.
27. Roth, D. B., Chang, X.-B. & Wilson, J. H. (1989) *Mol. Cell. Biol.* **9,** 3049–3057.