Conservative Intrachromosomal Recombination Between Inverted Repeats in Mouse Cells: Association Between Reciprocal Exchange and Gene Conversion

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ABSTRACT

Recombination in mammalian cells is thought to involve both reciprocal and nonreciprocal modes of exchange, although rigorous proof is lacking due to the inability to recover all products of an exchange. To investigate further the relationship between these modes of exchange, we have analyzed intrachromosomal recombination between duplicated herpes simplex virus thymidine kinase (HSV *tk*) mutant alleles arranged as inverted repeats in cultured mouse L cells. In crosses between inverted repeats, a single intrachromatid reciprocal exchange leads to inversion of the sequence between the crossover sites and recovery of both genes involved in the event. The majority of recombinant products do not display such inversion and are thus consistent with a nonreciprocal mode of recombination (gene conversion). The remaining products display the sequence inversion predicted for intrachromatid reciprocal exchange. In light of the fact that intrachromatid exchanges occur, the rarity of intrachromatid double reciprocal exchanges strengthens the interpretation that the majority of events in this and previous investigations involve gene conversion. Furthermore, in accord with prediction, one-third of the reciprocal recombinants (inversions) display associated gene conversion. This association suggests that reciprocal and nonreciprocal modes of exchange are mechanistically related in mammalian cells. Finally, the occurrence of inversion recombinants suggests that intrachromosomal recombination can be a conservative (nondestructive) process.

INTRACHROMOSOMAL recombination between closely linked repeated sequences in cultured mammalian cells has been studied using a variety of substrates (LISKAY and STACHELEK 1983, 1986; LIN and STERNBERG 1984; LISKAY, STACHELEK and LETSOU 1984; SMITH and BERG 1984; STRINGER *et al.* 1985; SUBRAMANI and RUBNITZ 1985; RUBNITZ and SUBRAMANI 1986). With substrates containing pairs of full-length mutant alleles (LISKAY and STACHELEK 1983; LISKAY, STACHELEK and LETSOU 1984; SMITH and BERG 1984; SUBRAMANI and RUBNITZ 1985), recombination can generate two types of products: one in which flanking markers are exchanged and one in which markers remain in the parental configuration. The former product can be interpreted as the result of a classical reciprocal crossover, while the latter resembles gene conversion, a nonreciprocal form of information transfer. These recombination events may involve two kinds of interactions: intrachromatid, in which all DNA transactions are intramolecular; or sister chromatid, in which events are precipitated by unequal pairing between replicated DNA duplexes.

Studies from this laboratory have involved extensive analysis of recombination between a single pair of full-length direct repeats (LISKAY, STACHELEK and LETSOU 1984; LETSOU and LISKAY 1986). A majority of these events (85%) do not perturb the external marker configuration and thus resemble gene conversion, while the remainder involve reciprocal flanking marker exchange. However, the nature of the interaction leading to recombination cannot be determined for recombinants recovered in these studies. A simple crossover between genes on the same molecule (intrachromatid) generates a product identical to that resulting from a crossover between unequally paired genes on sister chromatids. This product is a single reconstructed wild-type gene with the sequence between the points of exchange deleted. Likewise, conversion of one of the two alleles without flanking marker exchange leads to identical recombinants regardless of the nature of the interaction. An additional ambiguity is introduced by the possibility that double reciprocal exchange between sister chromatids following unequal pairing could produce recombinant products that are indistinguishable from single gene conversion events. It seems unlikely that double exchange events between sister chromatids are the most prevalent recombination events; however, we cannot exclude this explanation because of the difficulty in recovering all products of a recombination event.

Gene conversion as defined in fungi is a localized non-Mendelian segregation whereby information

from one of two interacting DNA molecules is replaced by corresponding information from its partner, with no change in the donor molecule. Rigorous proof for gene conversion is only possible in fungi, where meiotic analysis allows the recovery of all DNA strands involved in a recombination event. A common observation in such studies is that nonreciprocal recombination events (gene conversions) are often associated with reciprocal flanking marker exchange (for reviews see FOGEL *et al.* 1979; ORR-WEAVER and SZOSTAK 1985). Such an association is a common feature of recombination models (*e.g.*, MESELSON and RADDING 1975; SZOSTAK *et al.* 1983). Exceptions to this theme come from studies of meiotic recombination in Ascobolus (ROSSIGNOL *et al.* 1984), Drosophila (CARPENTER 1984), between repeated genes in yeast (KLEIN and PETES 1981; KLAR and STRATHERN 1984; KLEIN 1984; JACKSON and FINK 1981, 1985), and during mitotic growth in yeast (ROMAN and FABRE 1983), each study having demonstrated that the two types of recombination can be dissociated.

To expand the scope of our studies in mammalian cells and to better simulate meiotic analyses in fungi, we have studied recombination between full-length genes arranged as inverted repeats. Intrachromatid crossing over between inverted repeats is distinguishable from unequal sister chromatid exchange by flanking marker analysis. Intrachromatid crossing over leads to the recovery of both genes with an inversion of the sequence between the points of exchange; whereas products of a single unequal sister chromatid crossover are aberrant and not likely to be recovered. The two products of an intrachromatid event are thus available on a single chromsome for analysis (analogous to half-tetrad analysis in yeast). From these studies we have been able to: (1) obtain stronger evidence for nonreciprocal recombination than was possible using direct repeats, (2) establish a frequent association between gene conversion and reciprocal exchange, (3) determine that reciprocal events can occur either within a chromatid or between sister chromatids, and (4) provide strong evidence for a conservative mechanism of recombination for chromosomal sequences in mouse cells.

MATERIALS AND METHODS

Cell culture and generation of experimental lines: Thymidine kinase-deficient (tk^-) mouse L cells were cultured at 37° with 5% CO_2 in Dulbecco's modified Eagle media (DMEM) supplemented with 12% fetal bovine serum or with 2% fetal and 10% newborn bovine sera (Sigma). Cell lines were derived by introducing *Cla*I-linearized pJS-4 into nuclei by either of two methods: by calcium phosphate/DNA coprecipitation as described previously (LISKAY, STACHELEK and LETSOU 1984) or by direct microinjection (CAPECCHI 1980). Transformants were selected with 400 μg/ml G-418 sulfate (Geneticin, Gibco), subcloned, and tested for stability as described previously (LISKAY, LETSOU and STACHELEK 1987).

Plasmid description: Plasmid pJS-4 is identical to pJS-3 described previously (LISKAY, STACHELEK and LETSOU 1984), except that the 2.5-kb HSV *tk* gene inserted at a *Bam*HI site is flipped so that complementary sequences in the 2.5- and 2.0-kb segments are oriented in opposite directions (see Figure 1, "parent"). Note that the HSV *tk* DNA sequences are identical to those described previously, but sizes are reported to reflect more precisely the lengths based on the known *tk* gene sequence (WAGNER, SHARP and SUMMERS 1981 and W. C. SUMMERS, personal communication). The regions in which these sequences overlap are entirely homologous, except where *Xho*I linker insertions interrupt the coding sequence at positions 735 (*tk26*) and 1220 (*tk8*), according to the numbering system of WAGNER, SHARP and SUMMERS (1981). Mutations *tk26* and *tk8* are stable and have not been observed to revert spontaneously (frequencies less than 10^{-8}; LISKAY, STACHELEK and LETSOU 1984).

Southern transfer hybridization techniques: Cellular DNA was isolated and purified as previously described (LISKAY and EVANS 1980). Restriction enzymes were purchased from New England Biolabs and digestions were performed as recommended by the supplier. Southern transfer hybridization was performed essentially as previously described (LISKAY and STACHELEK 1983). Briefly, DNA restriction fragments (8 μg/lane) were separated by electrophoresis on 0.8% agarose gels (Sigma), denatured and transferred to nitrocellulose filters (Schleicher and Schuell). Filters were hybridized to 10^7 cpm of denatured HSV *tk* probe prepared by nick translation of the 2.5-kb *Bam*HI fragment of pJS-4 with [α-^{32}P]dCTP to a specific activity in excess of 10^8 cpm/μg using an Amersham nick translation kit.

Identification of low-copy parent lines: Copy number of plasmid integrations in parental lines was based on band intensities and on the number of unique junction fragments determined by hybridization techniques. DNAs were digested separately with *Hin*dIII or with *Bam*HI, liberating one gene on a fragment of predicted length and the other gene on a higher molecular weight junction fragment representing a cellular restriction site adjacent to the specific plasmid integration site. Copy numbers were verified by hybridization analysis of recombinants, in which only the wild-type gene is expected to become resistant to digestion with *Xho*I (for further discussion see LISKAY, LETSOU and STACHELEK 1987).

Recombination analysis: Recombination rates were determined by performing Luria-Delbrück fluctuation analyses on colonies arising in HAT media (10^{-4} M hypoxanthine, 2×10^{-6} M aminopterin, 1.5×10^{-5} M thymidine; SZYBALSKI, SZYBALSKA and RAGNI 1962). For each parent line at least ten independent subcultures, each derived from a small number of progenitor cells, were expanded in nonselective media and plated into HAT media (3×10^6 cells per 100-mm dish; at least 6×10^6 cells per subculture). After 12 to 16 days under selection, surviving colonies were fixed with methanol, stained and counted. Rates based on HAT-resistant (HAT^r) colonies from independent subcultures were calculated as previously described (LISKAY, LETSOU and STACHELEK 1987). In addition to subcultures for fluctuation analyses, smaller scale subcultures were grown in parallel and $0.5–1 \times 10^6$ cells plated into HAT media. Single HAT^r segregants were harvested from each subculture for hybridization analysis, thus ensuring that each recombinant analyzed arose by an independent event.

TABLE 1

Rates of recombination between inverted repeats

Parent line	Copy no.	Rate
1	1	1.2×10^{-6}
2	1	1.6×10^{-6}
3	1	0.5×10^{-6}
4	4	1.3×10^{-6}
Mean		1.2×10^{-6}

Rates of recombination in four lines harboring inverted duplications of mutant HSV *tk* alleles. Four parent lines were subjected to Luria-Delbrück fluctuation analyses as described in MATERIALS AND METHODS. The rate represents events/duplication/cell division. To compensate for increased copy number, the overall rate for line 4 was divided by four (for rationale, see LETSOU and LISKAY 1987).

TABLE 2

Analysis of inverted-repeat recombinants

	Conversion		Reciprocal	
Parent line	*tk8*	*tk26*	Associated	Simple
1	56	22	1[a]	4
2	46	22	1[b]	2
3	22	10	2[c]	2
Totals	124	54	4	8
% (n = 190)	94%		6%	

Tabulation of recombinant products in three single-copy parent lines. Identifications were made by molecular hybridization analyses and classification according to Figure 1. One recombinant not included in the table was consistent with either symmetric heteroduplex or with double reciprocal intrachromatid exchange ("double mutant" depicted in Figure 1; see text). Three additional recombinants were not easily interpreted by our analyses and are not included.

[a] "Reciprocal + conversion *tk8*."

[b] "Reciprocal + separated conversion."

[c] "Reciprocal + conversion *tk8*" and "reciprocal + conversion *tk26*."

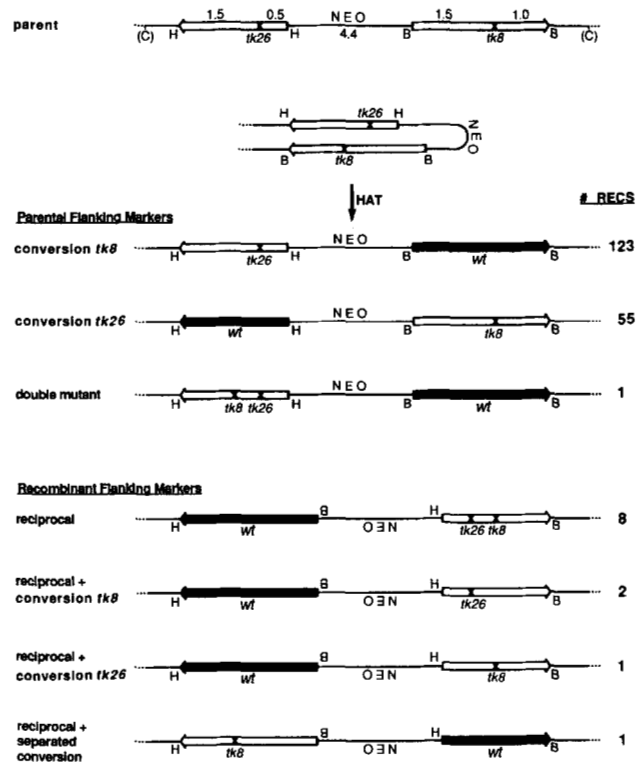

FIGURE 1.—Schematic representations of recombination substrate and recombination products selected in HAT media. Indicated are restriction sites for enzymes *Hin*dIII (H), *Bam*HI (B) and *Cla*I (C) in the original pJS-4 plasmid used to generate the cell lines in this study. *Arrowheads* represent directions of transcription of HSV *tk* sequences to emphasize the inverted nature of the duplication construct. The *tk* alleles flank the dominant selectable marker (NEO = resistance to G-418) used to generate cell lines. Alleles *tk8* and *tk26* are indicated at the site (×) of the *Xho*I linker insertion mutations. Among recombinants, wild-type genes, which no longer contain *Xho*I linker insertions, are shaded. Recombinants are separated into two categories according to flanking marker configurations (positions of *Hin*dIII and *Bam*HI sites). Products with parental flanking markers have unaltered junction fragments (established as in Figure 2) with one *tk* fragment flanked by *Hin*dIII sites and one fragment flanked by *Bam*HI sites, as in the "parent." Reciprocal recombinants have recombinant flanking markers (each *tk* fragment is flanked by *Hin*dIII and *Bam*HI sites) resulting from inversion of interstitial sequences, which is depicted by inverting NEO. Conversion products are identified by the loss of the *Xho*I restriction site marking a given allele (*e.g.*, allele *tk8* is lost in "conversion *tk8*"). Number of recombinants (#RECS) correspond to data tabulated in Table 2.

RESULTS

Rates of recombination: Four parent lines were generated which stably maintained duplicated mutant HSV *tk* genes arranged as inverted repeats (see Figure 1, "parent"). The basic recombination substrate was identical to that used in previous studies of direct repeats (LISKAY, STACHELEK and LETSOU 1984), except that the 2.5-kb fragment carrying the *tk8* allele flanked by *Bam*HI restriction sites was in the reversed configuration. These parent cell lines were subjected to Luria-Delbrück fluctuation analyses to determine rates of recombination between inverted repeats. The rates of recombination, shown in Table 1, displayed limited variation among these four parental lines. This is in accord with previous studies from our laboratory (LISKAY, LETSOU and STACHELEK 1987; LETSOU and LISKAY 1986, 1987), indicating that the recombination rate is intrinsic to a given construct and not heavily influenced by the site of integration in the L*tk*$^-$ cell genome. The rates for inverted repeats observed in this study (mean rate = 1.2×10^{-6}) were slightly lower than rates observed for the same pair of alleles arranged as direct repeats (mean rate = 3.6×10^{-6}; LISKAY, STACHELEK and LETSOU 1984; LETSOU and LISKAY 1986).

Analysis of recombinants. To deduce the nature of recombination events occurring between inverted

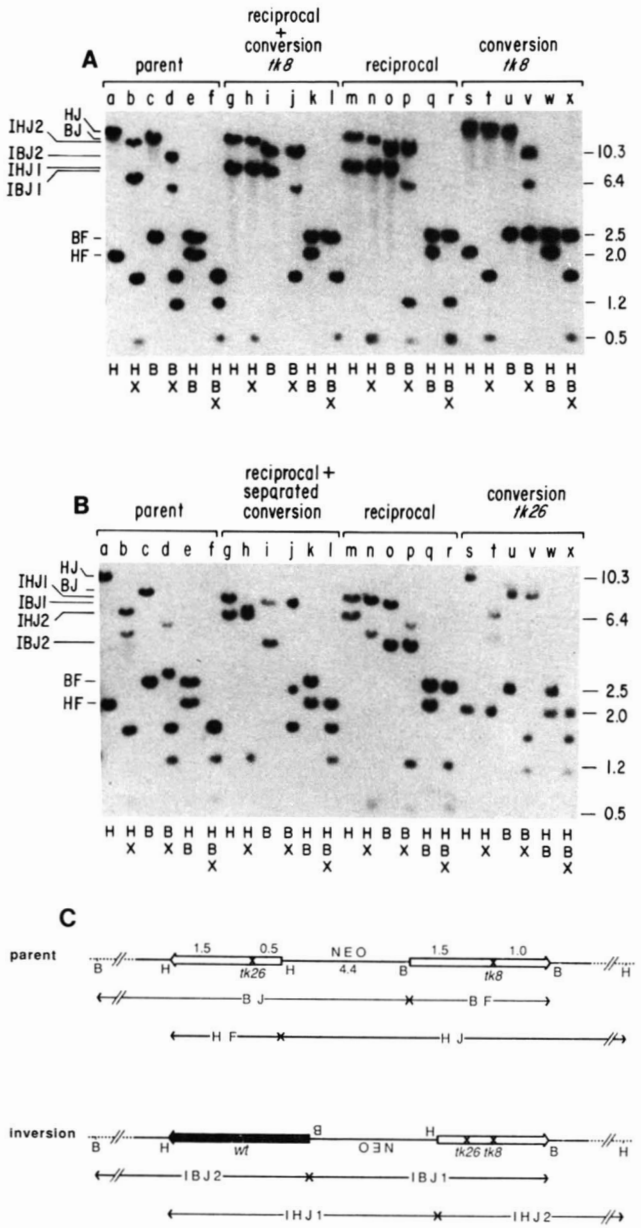

FIGURE 2.—Analysis of inverted-repeat recombinants. *A*, Molecular hybridization analysis of representative recombinants from parent line 1. DNAs from the HAT^s parent line and from the indicated HAT^r recombinants were digested with the enzymes indicated below each lane, separated by electrophoresis through agarose and probed with HSV *tk*-specific probe as described in MATERIALS AND METHODS. Restriction enzyme abbreviations are as in Figure 1. Examination of junction fragments provides flanking marker phenotype. The parent and conversion recombinants retain the same junction fragments (HJ for *Hin*dIII digestion, lanes a and s; BJ for *Bam*HI digestion, lanes c and u). Reciprocal recombinants possess novel junctions as predicted for inversion (IHJ1 and IHJ2 for *Hin*dIII digestion, lanes g and m; IBJ1 and IBJ2 for *Bam*HI digestion, lanes i and o; see interpretation of junctions in Figure 2C). Examination of resistance to *Xho*I digestion indicates wild-type *tk* sequence and sites of sequence correction. In "conversion *tk8*" (lanes s–x) the *Bam*HI fragment (BF, lanes u–x; sequences contained in HJ, lanes s, t) is resistant to digestion by *Xho*I, indicating that the *tk8* allele has been corrected to wild type, whereas the *Hin*dIII fragment (HF, lanes s and w; sequences contained in BJ, lane u) is sensitive to *Xho*I digestion, indicating the presence of only the *tk26* mutation by comparison with the "parent." In inversion recombinants [lanes g–r; depicted for simple inversion in (C)], wild-type sequences resistant to *Xho*I digestion are contained in the 2.5-kb fragment flanked by *Hin*dIII and *Bam*HI sites (IHJ1, IBJ2 and BF). The accompanying gene is sensitive to *Xho*I. In the case of simple "reciprocal" recombinants the accompanying gene (*e.g.*, on fragment IBJ1 in lane o) contains both alleles, *tk8* and *tk26*, and cuts twice with *Xho*I. There are three resultant bands in lanes n and r, but two of these comigrate on the gel at 0.5 kb (doublet; actual sizes 528 bp and 485 bp). By contrast in the "reciprocal + conversion *tk8*" the accompanying gene retains only the *tk26* allele and cuts only once to give identical fragments (lane l) as for the "conversion *tk8*" (lane x). Marker sizes at the right are given in kb. Fragment designations at the left are as described in (C). *B*, Molecular hybridization analysis of representative recombinants from parent line 2. The restriction digestions for each lane are the same as those in (A). Junction fragments are of different sizes for parent line 2 than for parent line 1 indicating a different site of integration in the genome. Flanking marker analysis is the same as in Figure 2A. In "conversion *tk26*" (lanes s–x) the 2.0-kb HF fragment has been corrected to wild type and does not cleave with *Xho*I (lanes s, t, w and x), while the 2.5-kb BF fragment retains only the *tk8* allele. Analysis of the simple "reciprocal" (lanes m–r) is the same as in (A). The recombinant labeled "reciprocal + separated conversion" (lanes g–l) is an inversion recombinant based on flanking marker analysis, but in contrast to the simple "reciprocal" discussed in *A*, the wild-type gene is contained in the 2.0-kb fragment flanked by *Hin*dIII and *Bam*HI sites (IHJ2, IBJ1 and HF). The accompanying gene possesses the *tk8* allele and cleaves once with *Xho*I to generate fragments (lane l) of sizes identical to those obtained with conversion *tk26* (lane x). For further discussion see the text. *C*, Interpretation of junction fragments for molecular hybridization analyses. Allele representations and notations are as described for Figure 1. Double bars represent unknown sequence between known plasmid sequences and the indicated cellular restriction sites, which vary among cell lines due to different sites of integration into the genome. Junction fragments are abbreviated as follows: HJ = *Hin*dIII junction; BJ = *Bam*HI junction; IHJ = inversion *Hin*dIII junction; IBJ = inversion *Bam*HI junction; BF = *Bam*HI fragment; HF = *Hin*dIII fragment. IBJ1 and IHJ1 are of constant size among parent lines (6.4 and 6.9 kb, respectively), while IBJ2 and IHJ2 vary among lines.

repeats, independent HAT^r segregants were analyzed by molecular hybridization techniques. In all cases the functional or wild-type *tk* gene could be identified by the diagnostic loss of a *Xho*I restriction site which marks the site of the linker insertion mutation. The majority of recombination products analyzed (178 of 190 or 94%; see Table 2) retained the accompanying gene in the parental configuration without gain of the *Xho*I mutation from the reconstructed wild type gene, as expected for gene conversion. Segregants which exhibited the 2.5-kb *Bam*HI fragment as wild type and retained the accompanying 2.0-kb *Hin*dIII fragment with the *tk26* mutation (as depicted in Figure 1) are classified as conversions of the *tk8* mutation in Table 2. Hybridization analysis of such a recombinant from parent line 1 is presented in Figure 2A, lanes s–x. Similarly, recombinants which had the 2.0-kb *Hin*dIII fragment as wild type and retained the *tk8* mutation in the accompanying 2.5-kb gene are classified as conversions of the *tk26* mutation (see Figure 1 and lanes s–x in Figure 2B).

A reproducible observation is that conversions of *tk8* occur roughly twice as often as conversions of *tk26* (124 conversions *tk8* to 54 conversions *tk26*; see Table 2). This discrepancy is consistent among all inverted repeat lines studied, but at present we have no explanation for this observation.

A second class of recombinants (12 of 190 or 6%), tabulated in Table 2 as reciprocal events, were consistent with an intrachromatid reciprocal exchange in that the 2.5-kb and 2.0-kb HSV *tk*-containing segments were each flanked by one *Hin*dIII site and one *Bam*HI site; *i.e.*, the flanking markers were exchanged. Individual *Bam*HI or *Hin*dIII digests of these recombinants demonstrated that junctions of novel sizes were now associated with each known restriction site (IBJ1 and IBJ2 in the case of *Bam*HI, IHJ1 and IHJ2 in the case of *Hin*dIII; see Figures 2A and 2B, lanes g–r and Figure 2C). Further restriction hybridization analyses on a number of these recombinants confirmed that these products were the result of an intrachromosomal reciprocal exchange between the two genes generating an inversion of chromosomal sequences between the crossover sites. These reciprocal exchanges most likely represent intrachromatid events, since single reciprocal exchanges involving unequally paired sister chromatids should generate unstable, aberrant chromsomes in which only the wild type gene has recombinant flanking markers (as depicted in Figure 3).

Reciprocal exchange associated with gene conversion: A subset of the reciprocal class of recombinants showing inversion of internal sequences also provided evidence for a nonreciprocal aspect to the recombination event. A single crossover in the interval between the two mutations without gene conversion at either mutant site would be expected to produce a viable wild type sequence on a 2.5-kb fragment flanked by *Hin*dIII and *Bam*HI sites. The accompanying 2.0-kb gene, also flanked by *Hin*dIII and *Bam*HI sites, would retain both mutations. Such products are termed simple reciprocal exchanges and are diagramed as "reciprocal" in Figure 1. However, 4 of 12 inversion products retained only one mutation in the nonfunctional gene, consistent with a nonreciprocal process at the other mutant site associated with the crossover. In two recombinants, the *tk8* mutation originally on the 2.5-kb fragment was lost, and in another case the *tk26* mutation originally on the 2.0-kb fragment was lost. An additional recombinant in the latter class was observed in line 4, but is not included in Table 2, since the complexity of line 4 (4 copies of the duplication) made accurate analysis of all recombinants difficult. These products, diagramed in Figure 1, are most easily explained by a reciprocal exchange with associated conversion of the *tk8* mutation ("reciprocal + conversion *tk8*"; molecular analysis presented in Figure 2A, lanes g–l) and reciprocal exchange with conversion of the

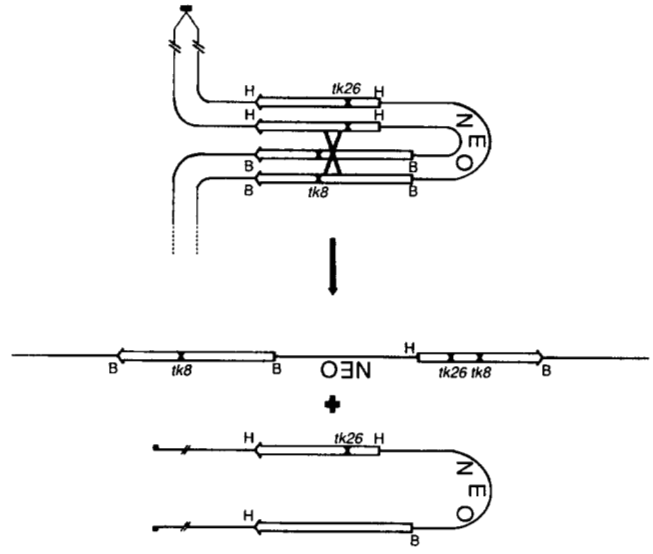

FIGURE 3.—Consequences of reciprocal exchange between inverted genes on unequally paired sister chromatids. Allele representations and notations are as described for Figure 1. Duplicated centromeres (*filled boxes*) are depicted in one of two possible orientations. As drawn, the wild-type gene would be generated on a dicentric chromatid with all centromere-proximal sequences duplicated and all sequences distal to the repeats deleted. In the reversed centromere configuration (not depicted), the wild-type gene would be generated on the acentric chromatid with all centromere-distal sequences duplicated and proximal sequences deleted. Neither chromatid is expected to segregate properly at mitosis. These products should be distinguishable from inversion recombinants, since only one allele displays recombinant flanking markers (H and B sites as indicated). As expected no such recombinants were recovered.

tk26 mutation ("reciprocal + conversion *tk26*"), respectively.

Simple crossovers and the associated events described above resulted in the wild type gene on a 2.5-kb fragment. In both these latter events, a gene conversion tract is presumed to have occurred adjacent to the crossover. One product which involved sequence inversion was recovered in which the 2.0-kb fragment was resistant to *Xho*I digestion and thus wild type ("reciprocal + separated conversion" in Figures 1 and 2B). The accompanying 2.5-kb fragment retained only the *tk8* mutation. Such a product is most easily explained by a conversion event at *tk26* separated from the crossover site as discussed below. Similar types of products have also been observed in experiments designed to recover recombinants between directly repeated genes (R. J. BOLLAG, J. L. STACHELEK and R. M. LISKAY, unpublished results).

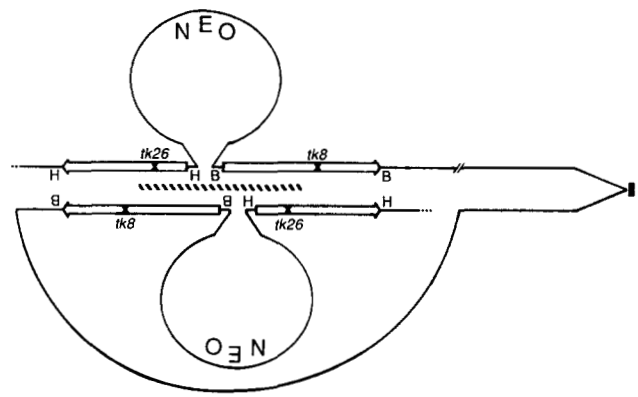

FIGURE 4.—Model for antiparallel pairing of sister chromatids across the region of the inverted repeats. Alleles are depicted as in Figure 1. Loops represent 4.4-kb sequences between *tk* alleles which are not in register in this pairing configuration. This mode of interaction as an alternative to intrachromatid exchange would require a conversion tract (or double crossovers) spanning the stippled region to generate the observed majority class of inversion recombinants ("simple reciprocal" in Table 2).

DISCUSSION

The results of this investigation show that homologous recombination between inverted repeats in mouse cells occurs at a rate of approximately 1.2×10^{-6}. This rate, albeit slightly reduced, is not markedly different from the rate of 3.6×10^{-6} observed with the same gene pair arranged as direct repeats (LISKAY, STACHELEK and LETSOU 1984; LETSOU and LISKAY 1986). The large majority of products seen here (94%) are consistent with a nonreciprocal recombination process in that flanking marker exchange (via inversion) was not seen. The remaining products can be explained by a reciprocal process (crossover) leading to flanking marker exchange and sequence inversion. One third of these crossovers were accompanied by gene conversion at one of the two mutant sites.

Among products of recombination between inverted repeats, approximately 6% (12 of 190) were consistent with intrachromatid reciprocal exchange, while none were consistent with unequal sister chromatid reciprocal exchange. This was not a surprising observation, since reciprocal exchange following unequal pairing of sister chromatids would lead in the case of inverted repeats to mitotically unstable chromosomes with duplications and deficiencies, as depicted in Figure 3. Studies of direct repeats suggest that 15% of all recombination events are reciprocal in nature, while the remaining events resemble gene conversion (LISKAY, STACHELEK and LETSOU 1984). In those studies, products of both sister chromatid and intrachromatid events could be recovered. The inability to recover products of sister chromatid events between inverted repeats may account for the reduced proportion of reciprocal exchanges observed in the present study as compared to those observed between direct repeats.

Although the simplest explanation for the inversion class of recombinants is a single intrachromatid crossover, there exists the formal possibility that these recombinants could arise following antiparallel mispairing of sister chromatids in the region of the duplication, such that both alleles on both chromatids are paired (depicted in Figure 4). Recombination following such pairing could be executed in either of two ways: by a gene conversion involving all four genes and including the 4.4 kb of nonhomology between the alleles or by double crossovers with one exchange in each gene pair. Both of these explanations require correction of a large nonhomology (*i.e.*, 4.4 kb). Meiotic conversions of large deletions and insertions occur in yeast and models have been detailed to account for these (*e.g.*, RADDING 1979). In recent yeast studies such intrachromosomal conversions spanning large nonhomologies have been proposed to account for "plasmid conversion" between duplicated *ADE8* alleles during meiosis (MALONEY and FOGEL 1987) and for mitotic rearrangements involving repeated delta sequences at the *SUP4* locus (ROTHSTEIN, HELMS and ROSENBERG 1987). However, recent results from our laboratory suggest that in mammalian cells, removal of insertions exceeding 1 kb occurs at least two orders of magnitude less efficiently than correction of small insertions of 1–8 bp (LETSOU and LISKAY 1987). Thus, we believe that the complex pairing configuration shown in Figure 4 is not likely to account for the observed inversions.

The predominant class of products in the present study (178 of 190 recombinants) appear to have arisen by a nonreciprocal mechanism which we term gene conversion. In these recombinants one gene has lost its mutant information with no corresponding change in the accompanying gene. Formally, such events

could have involved double exchanges between sister chromatids. We have no prior evidence for intrachromatid interactions in mammalian cells. Others have described recombination products between direct repeats consistent with unequal sister chromatid exchanges (SMITH and BERG 1984; STRINGER *et al.* 1985). The results of this study combined with those reported previously (LISKAY, STACHELEK and LETSOU 1984) suggest that both intrachromatid and sister chromatid interactions contribute to intrachromosomal recombination. If double exchange events rather than gene conversions are responsible for the major class of recombinants, then we should have frequently observed recombinants in which intrachromatid double exchanges resulted in a double mutant gene accompanying the wild type recombinant without sequence inversion. Among over 190 products examined, only one recombinant consistent with this interpretation was obtained (diagramed as "double mutant" in Figure 1). We prefer to explain this single event by symmetric heteroduplex covering the two mutations with independent correction on each chromatid, rather than as a double reciprocal intrachromatid exchange. Furthermore, events consistent with intrachromatid double exchanges were rarely (possibly one in greater than 200 total events) observed in previous studies with direct-repeat constructs (LISKAY, STACHELEK and LETSOU 1984; LISKAY and STACHELEK 1986; LISKAY, LETSOU and STACHELEK 1987; LETSOU and LISKAY 1987). Therefore, we conclude that the class of recombinants exhibiting no flanking marker exchange represent nonreciprocal events.

Further evidence for nonreciprocality in the present recombinant analysis is provided by the concomitant loss of one of the original mutations among one third of recombinants showing inversion (4 of 12 events). These products are most easily explained by a reciprocal exchange leading to inversion accompanied by nonreciprocal transfer of information at one of the two mutant sites. Since the overall frequency of all recombination events is approximately 10^{-5} it is unlikely that two independent events would be picked up in a single segregant. Therefore, we favor the notion that the two processes are associated and likely represent alternative resolutions to a single recombination event, as has been postulated for meiotic recombination in fungi (for a review see ORR-WEAVER and SZOSTAK 1985). If reciprocal exchange is always accompanied by gene conversion proceeding by heteroduplex formation at one of the two sites, and if correction at the mismatched site is unbiased (*i.e.*, restoration is as likely as conversion), then we would predict that one third of the total reciprocal events should display associated conversion. Our results are in accord with this prediction. Although our sample size is small and our knowledge of average conversion tract lengths in this system is limited (LISKAY and STACHELEK 1986), we conclude that most and possibly all reciprocal exchanges between repeated sequences in mouse cells are associated with nonreciprocal information transfer.

Among the inversions with associated conversion is included a recombinant depicted in Figures 1 and 2B as "reciprocal + separated conversion." This recombinant is difficult to explain by a single event involving conversion contiguous to the site of crossing over. Rather, our interpretation is that conversion occurred at the site of the *tk26* allele but the event was resolved as a crossover distal to the *tk8* allele. Similar reciprocal recombinants with separated conversion tracts have been observed in fungi, and possible explanations are discussed by WHITEHOUSE (1982) and by ORR-WEAVER and SZOSTAK (1985).

Studies by AYARES *et al.* (1985), in which extrachromosomal reciprocal recombinations between autonomously replicating plasmids generated dimers whereby both interacting molecules could be examined, yielded similar evidence for conversion associated with reciprocal exchange. However, extrachromosomal recombination frequencies are sufficiently high that such products may actually represent multiple events.

The ability to recover inversions in the present study also suggests that homologous recombination between chromosomal sequences in mammalian cells is a conservative process. CHAKRABARTI and SEIDMAN (1986) have reported that intramolecular recombination during transfection is nonconservative. They observed that when molecules bearing direct repeats undergo intramolecular interactions intended to yield reciprocal exchange extrachromosomally, the process generates only one of the two expected products. These authors suggested that one of the products suffered some destructive fate. Furthermore, intermolecular recombination events during transfection are rarely conservative (SEIDMAN 1987). The authors thereby conclude that most if not all extrachromosomal recombination proceeds via a nonconservative mechanism. In intrachromosomal recombination between direct repeats nonconservative events should be detected, because only one of two products need survive. Thus nonconservative and conservative processes could have contributed to the recombination observed between direct repeats (LETSOU and LISKAY 1986). In the present study, productive intrachromatid exchange to generate an inversion requires that the process be conservative to maintain the integrity of the chromosome. It should be noted that separate interactions involving both gene pairs following unequal pairing between sister chromatids (depicted in Figure 4) could generate the observed inversion in a process that need not be conservative. As discussed above, we feel that such an explanation

is unlikely. Because reciprocal events that are not necessarily conservative (between direct repeats) occur at only a slightly increased frequency over reciprocal events that are obligatorily conservative (between inverted repeats), we conclude that in contrast to extrachromosomal events, intrachromosomal recombination proceeds by a conservative mechanism. Earlier studies in our lab have suggested that extra- and intrachromosomal recombination differ mechanistically in other respects, such as a differential sensitivity to base-pair mismatch (WALDMAN and LISKAY 1987).

It is of interest to compare these results with those of similar studies in yeast and bacteria. Specifically, the proportions of reciprocal and gene conversion recombinants in these studies are similar to proportions of such events between duplicated *HIS4* alleles during mitotic recombination in yeast (JACKSON and FINK 1981). However, during meiotic recombination between repeated genes there is a marked deficit of conversions associated with reciprocal exchange (KLEIN and PETES 1981; KLAR and STRATHERN 1984; KLEIN 1984; JACKSON and FINK 1985). The similarity in frequencies of reciprocal recombination between inverted repeats (this study) and between direct repeats (LISKAY, STACHELEK and LETSOU 1984; LETSOU and LISKAY 1986) apparently does not hold in *Salmonella typhimurium* where inversion recombination occurs at lower frequencies than recombination between direct repeats (SEGALL and ROTH 1986).

This report presents results similar to those of WILLIS and KLEIN (1987) who, working with yeast, directly selected intrachromosomal inversions. These authors found that the inversions (*i.e.*, reciprocal exchanges) are frequently associated with gene conversion at one or more scorable sites within the repeats. Similarly, BORTS and HABER (1987) and SYMINGTON and PETES (1988) often found gene conversion in the vicinity of reciprocal exchanges during meiotic recombination in yeast. Association between gene conversion and reciprocal exchange is a common (FOGEL *et al.* 1979; ORR-WEAVER and SZOSTAK 1985) though not universal (KLEIN and PETES 1981; KLEIN 1984; JACKSON and FINK 1981, 1985; ROMAN and FABRE 1983; ROSSIGNOL *et al.* 1984) feature of fungal recombination. The frequent association has suggested that the two types of events are mechanistically related. This relationship is incorporated into the current models for recombination (MESELSON and RADDING 1975; SZOSTAK *et al.* 1983). Our study suggests that chromosomal recombination in mammalian cells shares this fundamental aspect of the mechanism with fungal recombination.

In summary, our studies using closely linked inverted repeats in mouse cells allow us to formulate several conclusions concerning intrachromosomal recombination. First, recombination between inverted repeats is similar in rate and products to recombination between direct repeats. Second, reciprocal exchanges can involve either intrachromatid or sister chromatid interactions. Third, reciprocal and nonreciprocal exchanges are frequently associated and are therefore likely to share a common mechanism. Finally our results argue that intrachromosomal recombination is a conservative process.

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LITERATURE CITED

AYARES, D., J. SPENCER, F. SCHWARTZ, B. MORSE and R. KUCHERLAPATI, 1985 Homologous recombination between autonomously replicating plasmids in mammalian cells. Genetics **111:** 375–388.

BORTS, R. H., and J. E. HABER, 1987 Meiotic recombination in yeast: alteration by multiple heterozygosities. Science **237:** 1459–1465.

CAPECCHI, M. R., 1980 High efficiency transformation by direct microinjection of DNA into cultured mammalian cells. Cell **22:** 479–488.

CARPENTER, A. T. C., 1984 Meiotic roles of crossing-over and of gene conversion. Cold Spring Harbor Symp. Quant. Biol. **49:** 23–29.

CHAKRABARTI, S., and M. M. SEIDMAN, 1986 Intramolecular recombination between transfected repeated sequences in mammalian cells is nonconservative. Mol. Cell. Biol. **6:** 2520–2526.

FOGEL, S., R. MORTIMER, K. LUSNAK and F. TAVARES, 1979 Meiotic gene conversion: a signal of the basic recombination event in yeast. Cold Spring Harbor Symp. Quant. Biol. **43:** 1325–1341.

JACKSON, J. A., and G. R. FINK, 1981 Gene conversion between duplicated genetic elements in yeast. Nature **292:** 306–311.

JACKSON, J. A., and G. R. FINK, 1985 Meioic recombination between duplicated genetic elements in *Saccharomyces cerevisiae*. Genetics **109:** 303–332.

KLAR, A. J. S., and J. N. STRATHERN, 1984 Resolution of recombination intermediates generated during yeast mating type switching. Nature **310:** 744–748.

KLEIN, H. L., 1984 Lack of association between intrachromosomal gene conversion and reciprocal exchange. Nature **310:** 748–753.

KLEIN, H. L., and T. D. PETES, 1981 Intrachromosomal gene conversion in yeast. Nature **289:** 144–148.

LETSOU, A., and R. M. LISKAY, 1986 Intrachromosomal recombination in mammalian cells. pp. 383–409. In: *Gene Transfer*, Edited by R. KUCHERLAPATI. Plenum, New York.

LETSOU, A., and R. M. LISKAY, 1987 Effect of the molecular nature of mutation on the efficiency of intrachromosomal gene conversion in mouse cells. Genetics **117:** 759–769.

LIN, F.-L., and N. STERNBERG, 1984 Homologous recombination between overlapping thymidine kinase gene fragments stably inserted into a mouse cell genome. Mol. Cell. Biol. **4:** 852–861.

LISKAY, R. M., and R. J. EVANS, 1980 Inactive *X* chromosome

DNA does not function in DNA-mediated cell transformation for the hypoxanthine phosphoribosyltransferase gene. Proc. Natl. Acad. Sci. USA **77:** 4895–4898.

LISKAY, R. M., and J. L. STACHELEK, 1983 Evidence for intrachromosomal gene conversion in cultured mouse cells. Cell **35:** 157–165.

LISKAY, R. M., and J. L. STACHELEK, 1986 Information transfer between duplicated chromosomal sequences in mammalian cells involves contiguous regions of DNA. Proc. Natl. Acad. Sci. USA **83:** 1802–1806.

LISKAY, R. M., A. LETSOU and J. L. STACHELEK, 1987 Homology requirement for efficient gene conversion between duplicated chromosomal sequences in mammalian cells. Genetics **115:** 161–167.

LISKAY, R. M., J. L. STACHELEK and A. LETSOU, 1984 Homologous recombination between repeated chromosomal sequences in mouse cells. Cold Spring Harbor Symp. Quant. Biol. **49:** 183–189.

MALONEY, D. H., and S. FOGEL, 1987 Gene conversion, unequal crossing-over and mispairing at a non-tandem duplication during meiosis of *Saccharomyces cerevisiae*. Curr. Genet. **12:** 1–7.

MESELSON, M. S., and C. M. RADDING, 1975 A general model for genetic recombination. Proc. Natl. Acad. Sci. USA **72:** 358–361.

ORR-WEAVER, T. L., and J. W. SZOSTAK, 1985 Fungal recombination. Microbiol. Rev. **49:** 33–58.

RADDING, C. M., 1979 The mechanism of conversion of deletions and insertions. Cold Spring Harbor Symp. Quant. Biol. **43:** 1315–1316.

ROMAN, H., and F. FABRE, 1983 Gene conversion and associated reciprocal recombination are separable events in vegetative cells of *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **80:** 6912–6916.

ROSSIGNOL, J.-L., A. NICOLAS, H. HAMZA and T. LANGIN, 1984 Origins of gene conversion and reciprocal exchange in *Ascobolus*. Cold Spring Harbor Symp. Quant. Biol. **49:** 13–21.

ROTHSTEIN, R., C. HELMS and N. ROSENBERG, 1987 Concerted deletions and inversions are caused by mitotic recombination between delta sequences in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **7:** 1198–1207.

RUBNITZ, J., and S. SUBRAMANI, 1986 Extrachromosomal and chromosomal gene conversion in mammalian cells. Mol. Cell. Biol. **6:** 1608–1614.

SEIDMAN, M. M., 1987 Intermolecular homologous recombination between transfected sequences in mammalian cells is primarily nonconservative. Mol. Cell. Biol. **7:** 3561–3565.

SEGALL, A. M., and J. R. ROTH, 1986 Restriction on the formation of spontaneous inversion rearrangements in *Salmonella typhimurium*. Genetics **113:** s14.

SMITH, A. J. H., and P. BERG, 1984 Homologous recombination between defective *neo* genes in mouse 3T6 cells. Cold Spring Harbor Symp. Quant. Biol. **49:** 171–181.

STRINGER, J. R., R. M. KUHN, J. L. NEWMAN and J. C. MEADE, 1985 Unequal homologous recombination between tandemly arranged sequences stably incorporated into cultured rat cells. Mol. Cell. Biol. **5:** 2613–2622.

SUBRAMANI, S., and J. RUBNITZ, 1985 Recombination events after transient infection and stable integration of DNA into mouse cells. Mol. Cell. Biol. **5:** 659–666.

SYMINGTON, L. S., and T. D. PETES, 1988 Expansions and contractions of the genetic map relative to the physical map of yeast chromosome *III*. Mol. Cell. Biol. **8:** 595–604.

SZOSTAK, J. W., T. L. ORR-WEAVER, R. J. ROTHSTEIN and F. W. STAHL, 1983 The double-strand-break repair model for recombination. Cell **33:** 25–35.

SZYBALSKI, W., E. H. SZYBALSKA and G. RAGNI, 1962 Genetic studies with human cell lines. Natl. Cancer Inst. Monogr. **7:** 75–88.

WAGNER, M. J., J. A. SHARP and W. C. SUMMERS, 1981 Nucleotide sequence of the thymidine kinase gene of herpes simplex virus type 1. Proc. Natl. Acad. Sci. USA **78:** 1441–1445.

WALDMAN, A. S., and R. M. LISKAY, 1987 Differential effects of base-pair mismatch on intrachromosomal versus extrachromosomal recombination in mouse cells. Proc. Natl. Acad. Sci. USA **84:** 5340–5344.

WHITEHOUSE, H. L. K., 1982 *Genetic Recombination: Understanding the Mechanisms*. John Wiley & Sons, New York.

WILLIS, K. K., and H. L. KLEIN, 1987 Intrachromosomal recombination in *Saccharomyces cerevisiae*: reciprocal exchange in an inverted repeat and associated gene conversion. Genetics **117:** 633–643.

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