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Direct-Repeat Analysis of Chromatid Interactions during Intrachromosomal Recombination in Mouse Cells

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Homologous intrachromosomal recombination between linked genes can involve interactions that are either intramolecular (intrachromatid) or intermolecular (sister chromatid). To assess the relative proportions of chromatid interactions, we report studies of intrachromosomal recombination in mouse L cells containing herpes simplex virus thymidine kinase genes in two alternative configurations of direct repeats. By comparing products of reciprocal exchanges between these two configurations, we conclude that the majority of interactions that give rise to crossover products involve unequally paired sister chromatids after DNA replication. Analyses of an additional class of crossover products that involve discontinuous associated gene conversion suggest that these recombination events involve a heteroduplex DNA intermediate.

Homologous intrachromosomal recombination between closely linked sequences can result in genetic rearrangements such as deletions, duplications, or inversions (2). Detectable products of intrachromosomal recombination can involve either intrachromatid interactions between linked sequences on a DNA molecule or sister chromatid interactions between unequally paired homologous sequences on sister strands following DNA replication (Fig. 1). This study examines the relative proportions of intrachromatid and sister chromatid interactions in mouse L cells.

Although gene conversion, a nonreciprocal transfer of information between homologous sequences, is the predominant mode of intrachromosomal recombination in mouse L cells (14), it is not possible to distinguish between convertants derived from sister chromatid and intrachromatid interactions. On the other hand, reciprocal exchanges, or crossovers, often generate products for which the type of interaction can be ascertained. For instance, reciprocal exchanges between direct repeats can lead to gene deletion (13, 14, 27). Through recombination involving unequally paired sister chromatids, reciprocal exchanges between direct repeats can generate increased numbers of genes (8, 13, 23, 26, 27), thereby providing a mechanism for gene amplification (22, 25). Moreover, intrachromatid reciprocal exchanges between closely linked genes in an inverted orientation can result in sequence inversion (1, 27). Whereas both intrachromatid and sister chromatid crossovers have been documented, there has been no reported study to measure systematically the relative proportions of intrachromatid and sister chromatid interactions in mammalian cells.

To address this question, we analyzed recombination between alternative configurations of direct repeats in cultured mouse cells. As depicted in Fig. 2 and 3, a simple intrachromatid crossover between direct repeats should generate two products: one hybrid gene contained on a circular DNA molecule and the reciprocal product, a hybrid gene remaining in the chromosome. During cell propagation, the circular DNA molecule should be lost unless it reintegrates into the genome. Therefore, the surviving product of an intrachromatid crossover is a chromosome bearing a single gene; the second gene and the interstitial sequence are deleted. A sister chromatid crossover between a pair of direct repeats produces one chromatid with a triplication and one chromatid with only a single gene. Chromosomes with only a single gene, whether arising through sister chromatid or intrachromatid interactions, are indistinguishable and are designated deletion products in this study. On the other hand, triplications are assumed to result from a sister chromatid interaction. Since in our system recombinants are identified by selection for thymidine kinase (TK) activity (14), which reciprocal product harbors the wild-type gene (i.e., deletion or triplication chromosome) depends on the orientations of mutations within the genes (Fig. 2 and 3).

Orientation I, as originally designated by Jackson and Fink (7), refers to a configuration in which mutations are proximal to the interstitial sequences, while orientation II refers to genes with mutations located distal to the interstitial sequences (see also a study by Klein [9]). Simple reciprocal recombination (i.e., not associated with gene conversion) between direct repeats in orientation I generates deletion products regardless of the nature of the exchange (summarized in Fig. 4). By contrast, sister chromatid crossovers between orientation II direct repeats result in triplication recombinants, while products of simple intrachromatid exchange are not recovered (summarized in Fig. 4). A comparison of the products of recombination obtained from these two direct-repeat orientations provides information on the proportions of intrachromatid and sister chromatid interactions and forms the basis of this study. By comparing the observed frequencies and types of reciprocal exchanges obtained in each orientation, we have evaluated the relative proportions of sister chromatid and intrachromatid reciprocal exchanges occurring during intrachromosomal recombination in mouse L cells.

MATERIALS AND METHODS

Cell culture and generation of experimental lines. TK-deficient (tk^-) mouse L cells were cultured at 37°C with 5% CO_2 in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum or with 2% fetal and 10%

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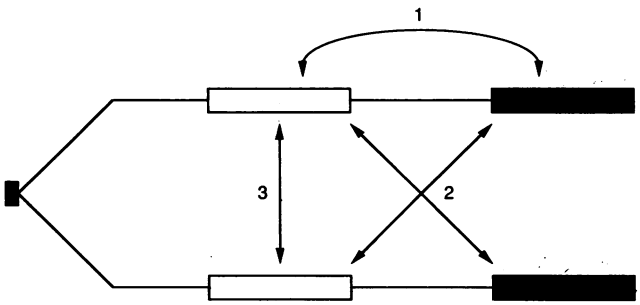

FIG. 1. Types of chromatid interactions. Diagramed are sister chromatids with pairs of repeated genes (blocks). (1) Intrachromatid exchange between linked genes on one chromatid. Interactions of this type can occur at the two-strand stage (i.e., before DNA replication) or at the four-strand stage (i.e., after DNA replication). (2) Unequal sister chromatid recombination between genes at nonallelic positions on sister chromatids. Such interactions require unequal pairing and can occur only at the four-strand stage. (3) Equal sister chromatid recombination between identical sequences at allelic positions. Such interactions, if they occur, have no genetic consequence in our recombination studies and go undetected.

newborn bovine sera (Sigma). Cell lines containing the recombination substrate were derived by introducing *Cla*I-linearized plasmids into nuclei by either of two methods: by calcium phosphate-DNA coprecipitation as described previously (14) or by direct microinjection (3). Transformants were selected with G-418 sulfate (Geneticin; 400 μg/ml; GIBCO), subcloned, and tested for stability as described previously (12).

Plasmids. Plasmid pRB-1 (the orientation II direct-repeat substrate) is identical to pJS-3 (the orientation I direct-repeat substrate) described previously (14) except for the reversal in orientation of the recombining genes. The 2.5-kb fragment in the *Bam*HI site, containing the herpes simplex virus *tk8* allele (interrupted by *Xho*I linkers at nucleotide position 1220 in the coding sequence, numbered according to reference 30), and the 2.0-kb fragment in the *Hin*dIII site, containing the *tk26* allele (*Xho*I linker at nucleotide position 735), are flipped at their respective sites. In both orientations, *tk8* and *tk26* are transcribed in the same direction.

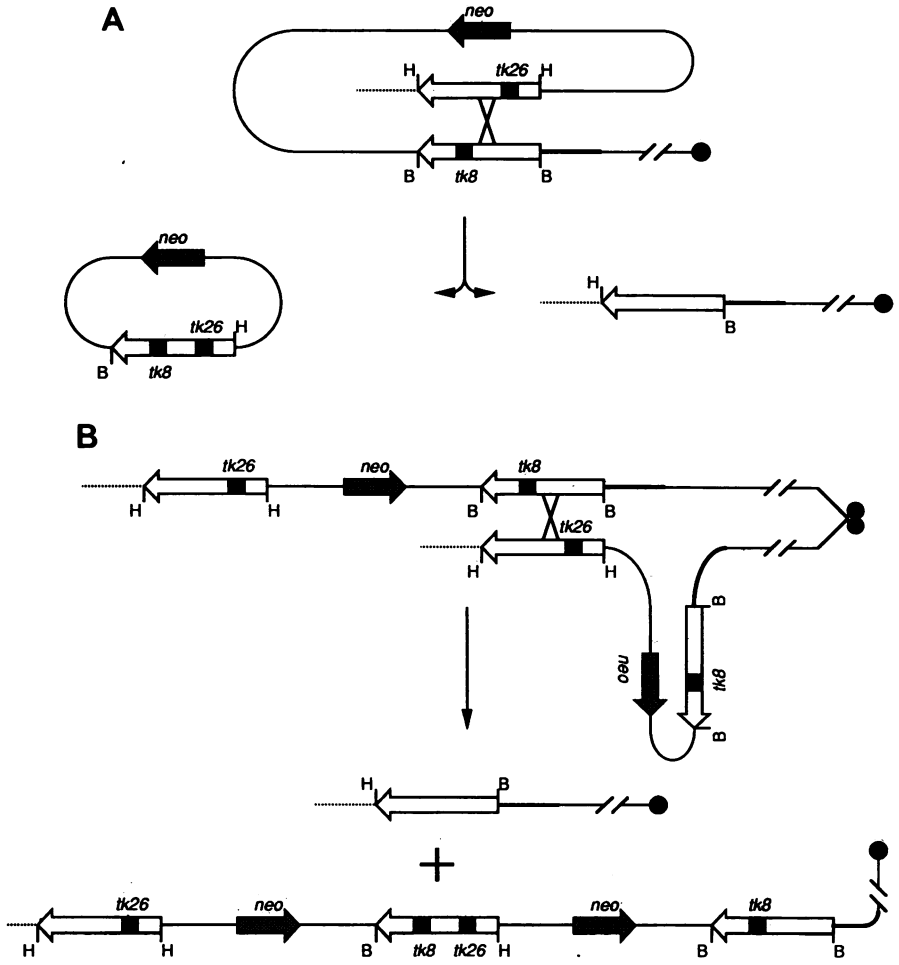

FIG. 2. Simple reciprocal exchange between orientation I direct repeats. *Xho*I linker insertion mutations *tk26* and *tk8* as indicated are proximal to a central *neo* gene. (A) Products of an intrachromatid crossover. H, *Hin*dIII restriction sites flanking the *tk26* allele; B, *Bam*HI restriction sites flanking the *tk8* allele. Simple reciprocal exchange in the region between the mutations leads to a wild-type sequence in the chromosome with looping out (pop-out) of the double-mutant gene and *neo*. Reciprocal products have hybrid flanking markers, *Hin*dIII and *Bam*HI. (B) Sister chromatid crossover. Centromere location is hypothetical; the relative orientation of the centromere is inconsequential. Simple reciprocal exchange (i.e., not associated with gene conversion) between *tk8* and *tk26* alleles on either strand generates a single wild-type gene on one chromatid and a double-mutant gene flanked by two mutant genes on the sister chromatid that is lost. Deletion products of either intrachromatid or sister chromatid crossovers are indistinguishable, and both render the cell $G418^s$.

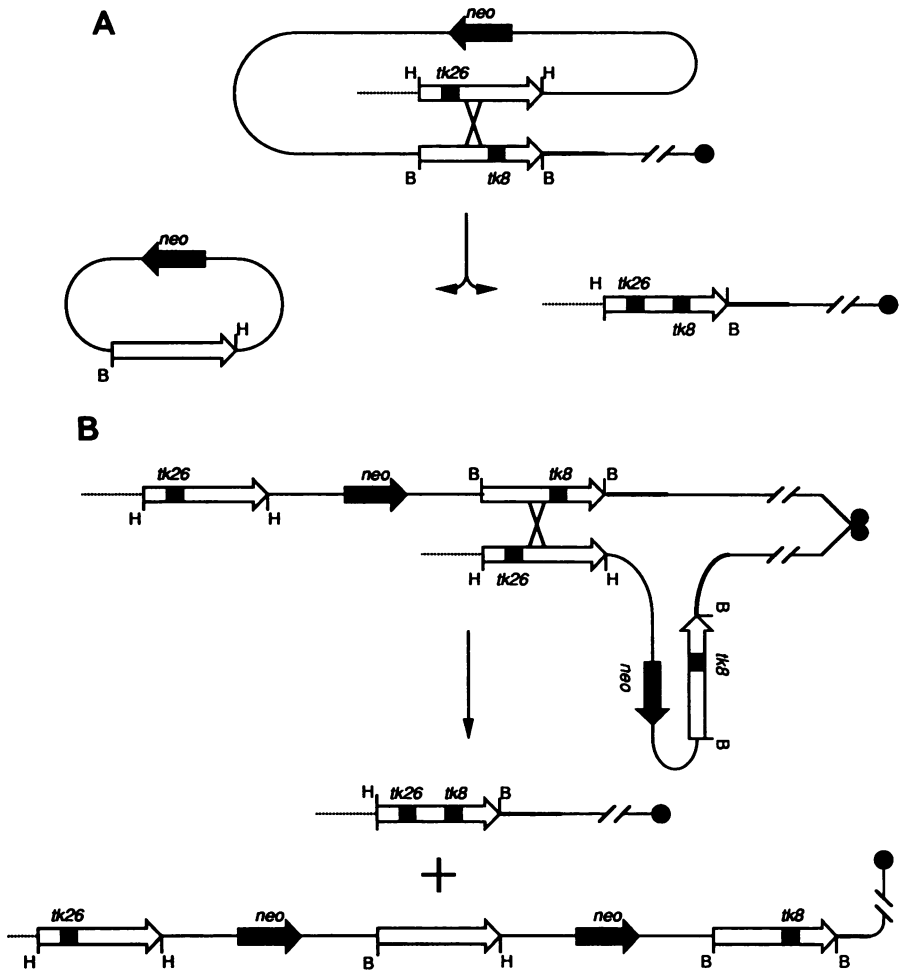

FIG. 3. Simple reciprocal exchange between orientation II direct repeats. Mutations *tk8* and *tk26* are distal to a central *neo* gene. (A) Products of an intrachromatid crossover leading to looping out (pop-out) of the wild-type gene and generation of a double-mutant gene in the chromosome. The circular pop-out product is not recovered; intrachromatid reciprocal exchange is not productive except in the special case diagramed in Fig. 6. (B) Sister chromatid crossover generating on one chromosome a wild-type gene flanked by two mutant genes, resulting in a triplication product; the reciprocal product harbors a double-mutant gene on a deletion chromosome.

Mutations *tk26* and *tk8* are stable and revert spontaneously at frequencies less than 10^{-8} (14).

Southern transfer hybridization techniques. Cellular DNA was isolated and purified as previously described (12). Restriction enzymes were purchased from New England Bio-Labs, and digestions were performed as recommended by the supplier. Southern transfer hybridization was performed essentially as previously described (12). Briefly, DNA restriction fragments (8 μg per lane) were separated by electrophoresis on 0.8% agarose gels (Sigma), denatured, and transferred to nitrocellulose filters (Schleicher & Schuell). Filters were hybridized to 10^7 cpm of denatured herpes simplex virus *tk* probe prepared by nick translation of the 2.5-kb *Bam*HI fragment of pJS-3 with [α-^{32}P]dCTP to a specific activity in excess of 10^8 cpm/μg, using an Amersham nick translation kit.

Identification of single-copy parent lines. Single-copy parent lines were initially screened by examination of DNA flanking the insertion site by hybridization analysis and confirmed by hybridization analysis of individual TK$^+$ recombinants. 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 single higher-molecular-weight junction fragment created by cleavage at a cellular restriction site adjacent to the specific plasmid integration site. Single-copy integration was verified by hybridization analysis of recombinants, in which the fragment containing the wild-type gene became resistant to digestion with *Xho*I.

Recombination analysis. Recombination rates were determined by performing Luria-Delbrück fluctuation analyses on colonies arising in HAT medium (100 μM hypoxanthine, 2 μM aminopterin, 15 μM thymidine). For each parent line, at least 10 independent subcultures, each derived from a small number of progenitor cells, were expanded in nonselective medium and plated into HAT medium (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^r colonies from independent subcultures were calculated as previously described (12). In addition to subcultures for fluctuation analyses, smaller-scale subcultures were grown in parallel and 0.5×10^6 to 1×10^6 cells were plated into HAT medium. Single HAT^r segregants were harvested from each subculture for hybridization analysis, thus ensuring

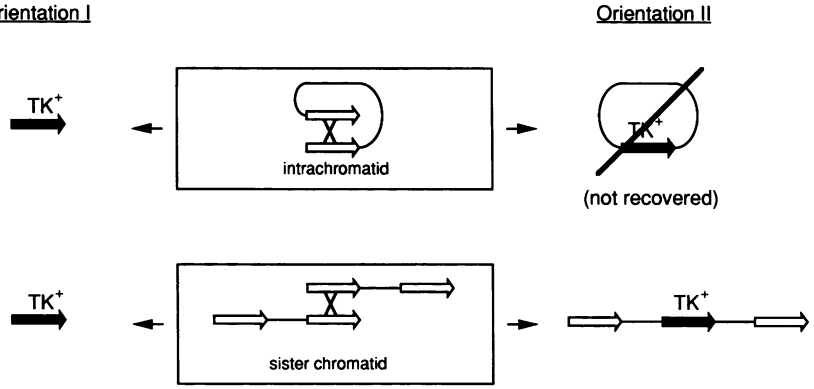

FIG. 4. Rationale for direct-repeat analysis of chromatid interactions. The diagram shows predicted products of intrachromosomal reciprocal exchanges between direct repeats; only products containing wild-type *tk* genes (indicated in black) are depicted. Simple reciprocal recombination between direct repeats in orientation I generates deletion products regardless of the nature of the exchange. By contrast, sister chromatid reciprocal exchanges result in triplication recombinants between orientation II direct repeats, while products of simple intrachromatid crossovers (not associated with gene conversion) are not recovered. Hence, SCR = Ori II/Ori I, where SCR is the frequency of sister chromatid reciprocal exchanges between direct repeats, Ori I represents (deletions in orientation I)/(total recombination products in orientation I), and Ori II represents (triplications in orientation II)/(total recombination products in orientation II). It should be noted that SCR may be slightly overestimated, since Ori II would be lower by the deficit of intrachromatid exchanges that are inviable. However, the magnitude of this overestimation should be minimal, since reciprocal exchanges represent only 17% of all products (Table 3), while gene conversions predominate (14).

that each recombinant analyzed arose by an independent event.

RESULTS AND DISCUSSION

The system for our direct-repeat analysis has been described previously (14). Two herpes simplex virus *tk* genes, mutant by virtue of *Xho*I linker insertions at separate sites in the coding region, are arranged in direct-repeat configuration. In orientation I, allele *tk8* is transcribed toward allele *tk26*, whereas in orientation II, *tk26* is transcribed toward *tk8*. We isolated tk^- L cells containing recombination substrates by selection for a bacterial neomycin resistance gene (*neo*) located between the two alleles. Intrachromosomal recombination generated a functional *tk* gene, which allowed TK^+ cells to survive in HAT medium. We isolated independent recombinants and studied both rates of recombination and types of recombinants by genetic and physical analyses.

As discussed in the introduction, a comparison of reciprocal recombinants obtained from each orientation should provide a measure of the relative contributions of intrachromatid and sister chromatid interactions. Taking into account only reciprocal exchange products, both sister chromatid and intrachromatid crossovers should result in TK^+ colonies in orientation I, while only sister chromatid crossovers should generate TK^+ segregants in orientation II (Fig. 4). Orientation I deletions provide an estimate of reciprocal exchanges occurring between the sites of *tk8* and *tk26*, while orientation II triplications reflect only the sister chromatid crossovers in the same region. Hence, the ratio of the fraction of triplications in orientation II to the fraction of deletions in orientation I should indicate the proportion of sister chromatid reciprocal recombination between direct repeats, as described in the equation in the legend to Fig. 4.

To determine whether the comparison of orientation I with orientation II cell lines is valid, rates of recombination for the two orientations were measured (Table 1). Rates from orientation I are taken from Letsou and Liskay (11), except in the case of line 3-3-3-M that was retested in this investigation. All cell lines used in this study had only a single copy of the recombination substrate. The mean rates for orientations I and II, determined to be 2.8×10^{-6} and 1.8×10^{-6}, respectively, are not significantly different on the basis of Student's unpaired *t* test ($P < 0.05$). Thus, we compared recombination between genes in these two orientations further.

Products of recombination were analyzed in two ways: by a genetic assay involving analysis of G418 sensitivity and by molecular hybridization analysis. In single-copy cell lines, deletion products eliminate the interstitial *neo* gene and render the recombinants $G418^s$. Hence, the percentage of $G418^s$ colonies represents the proportion of deletion recombinations. Results from the G418 analysis are presented in Table 2; percentages were corrected so as to include only independent recombination events.

The majority of recombinants analyzed by Southern blot hybridization could be classified in four categories. Analysis of representative recombinants from each class for an orientation II direct-repeat line is presented in Fig. 5; a similar analysis of products from orientation I lines has been presented previously (14). $G418^s$ recombinants constituted one

TABLE 1. Rates of recombination between direct repeats

Orientation	Cell line[a]	Rate (10^{-6})[b]
I	3-10[c]	3.5
	3-3-3-M	2.4
	3-3-4[c]	2.6
	Mean	2.8
II	3A-1	1.3
	C-1	1.9
	I-1-1	4.0
	E-3-1	1.4
	5-1-2	0.4
	Mean	1.8

[a] All lines harbor a single pair of *tk* alleles stably integrated into the genome.
[b] Recombinations per cell per generation.
[c] Data from reference 11.

TABLE 2. G418 phenotypes of recombinants from direct repeats

Orientation	Cell line	No. of colonies tested[a]	% G418[s]
I	3-3-3-M[b]	72	15
	3-10[b]	25	24
	Total	97	17
II	3A-1	80	5
	C-1	68	3
	I-1-1	20	0
	E-3-1	19	3
	5-1-2	14	0
	Total	201	3

[a] Averages from two pools of recombinants.
[b] Data combined with those from reference 14.

class, designated deletion recombinants in this study. Molecular analysis of genomic DNA fragments from this class cleaved with endonuclease *Hin*dIII revealed only a single band hybridizing to the *tk* probe (Fig. 5). In contrast to the parent, the *tk* gene within this band does not cleave with *Xho*I.

G418^r recombinants fell into the remaining three categories. One class had an unaltered *tk26* allele but was converted to wild type at the *tk8* allele (conversion *tk8*), and an analogous class had an unaltered *tk8* allele but was converted to wild type at the *tk26* allele (conversion *tk26*). These two classes are considered gene conversions (2, 14). Two *tk*-hybridizing bands were present in *Hin*dIII-restricted DNA; the upper band was resistant to *Xho*I digestion in the case of gene conversion of *tk8*, while the lower (2.0-kb band bounded by *Hin*dIII sites) was resistant to *Xho*I digestion in conversions of *tk26* (Fig. 5).

The final class of G418^r recombinants, observed only in orientation II lines, consisted of recombinants with three *tk*-hybridizing fragments in *Hin*dIII-restricted DNA. Two of the bands were identical to those of the parental DNA, but the third band was of novel length and proved to be *Xho*I resistant, indicating that it contained the wild-type gene (Fig. 5). These recombinants were classified as triplications and presumably arose through reciprocal recombination between unequally paired sister chromatids.

The results of the molecular hybridization analyses are presented in Table 3. Gene conversions constituted the majority of recombinants in orientation II (79.6%), in good agreement with analyses of orientation I direct-repeat recombinants (82.6%; Table 3 [14]) and inverted repeats (94% [1]). Reciprocal exchanges involving sister chromatids (generating triplications) constituted 17% of all products in the orientation II direct-repeat lines. Orientation I reciprocal exchange products (deletions) that could have resulted from either sister chromatid or intrachromatid interactions also constituted 17% of all recombinants.

From the similarity of these two percentages, we deduce that the majority of products from direct repeats arose through sister chromatid rather than intrachromatid interactions. Hence, on the basis of the analysis summarized in Fig. 4, we conclude that recombination between unequally paired sister chromatids (pathway 2 in Fig. 1) predominates over recombination within a chromatid (pathway 1 in Fig. 1). Solely on the basis of the results of our study, there is no need to invoke intrachromatid reciprocal exchange to explain direct-repeat reciprocal recombinants. However, previous studies with inverted repeats provide evidence that

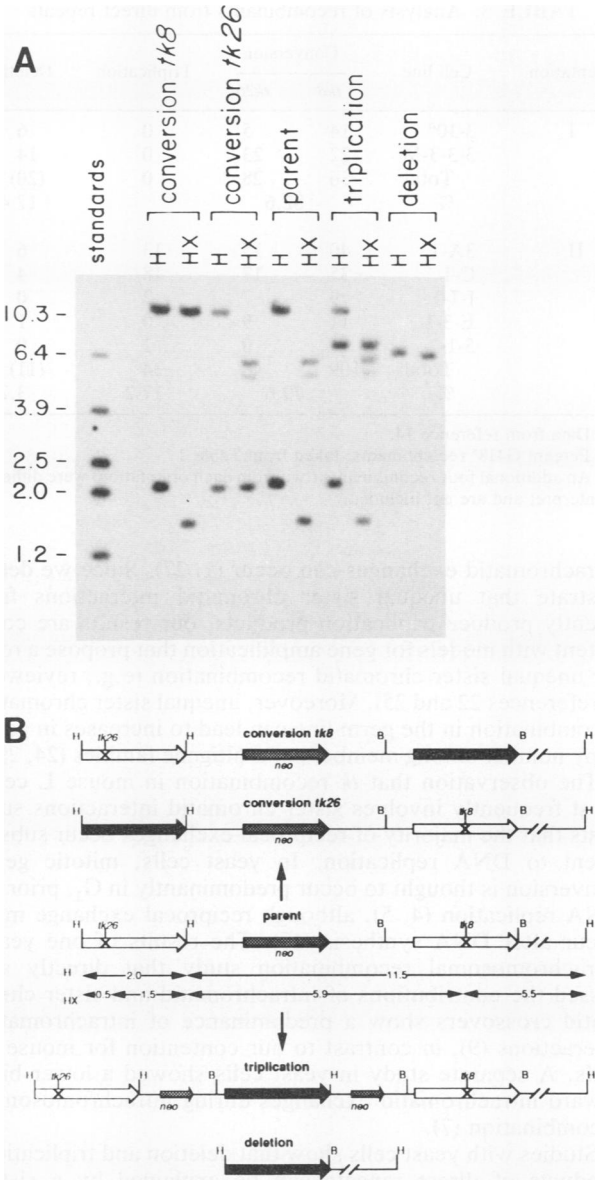

FIG. 5. Gene number changes in direct-repeat recombination. (A) Analysis of orientation II direct-repeat recombinants as depicted in panel B. Gene conversions (left of parent) do not result in changes in gene copy number. Reciprocal exchanges (right of parent) result in duplication or deletion of one gene partner. Recombinants and parent are from cell line C-1, and each DNA is digested with *Hin*dIII alone (H) or with both *Hin*dIII and *Xho*I (HX). The parent has two *tk*-hybridizing bands liberated by *Hin*dIII digestion, a 2.0-kb band reflecting original *Hin*dIII sites on pRB-1 and a high-molecular-weight fragment reflecting the next *Hin*dIII site at this integration position. In the parent, both bands are cleaved with *Xho*I in the HX lane. All recombinants examined possess a *Xho*I-resistant (wild-type) gene. For the majority of recombinants, either the 2.0-kb fragment (possessing the *tk26* allele) or the junction fragment (possessing the *tk8* allele) is resistant to digestion with *Xho*I. These are classified in Table 3 as gene conversions of *tk26* or of *tk8*, respectively. In triplication products, which most likely result from crossovers between unequally paired sister chromatids, there is a novel *Xho*I-resistant fragment of size 6.9 kb that contains a hybrid gene flanked by *Hin*dIII and *Bam*HI sites 2.5 kb apart. Deletion recombinants cannot be explained by simple reciprocal exchange. Such recombinants are most likely reciprocal exchanges with separated gene conversion (see text for discussion).

TABLE 3. Analysis of recombinants from direct repeats

Orientation	Cell line	Conversion		Triplication	Deletion
		tk8	*tk26*		
I	3-10[a]	14	5	0	6
	3-3-3-M	22	23	0	14
	Total	36	28	0	(20)
	%	82.6			17.4[b]
II	3A-1	40	15	13	6
	C-1	35	17	18	4
	I-1-1	9	7	2	0
	E-3-1	11	9	0	1
	5-1-2	14	0	1	0
	Total[c]	109	48	34	(11)
	%	79.6		17.2	3.2[b]

[a] Data from reference 14.
[b] Percent G418[s] recombinants; taken from Table 2.
[c] An additional four recombinants (two from each orientation) were difficult to interpret and are not included.

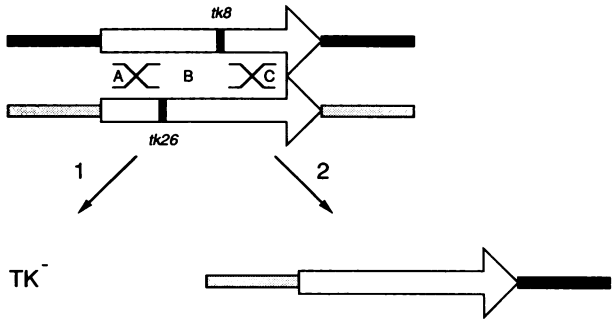

FIG. 6. Schematic representation of the product of reciprocal exchange accompanied by separated gene conversion. Flanking sequences are distinguished for the two alleles (filled for *tk8*; stippled for *tk26*). For orientation II direct repeats, simple reciprocal exchange in region B generates the products depicted in Fig. 3. Intrachromatid reciprocal exchange of this type is represented by pathway 1, but no products are recovered (TK^-). The wild-type product with flanking markers as depicted for pathway 2 cannot be generated by simple reciprocal exchange. This product can be generated either by reciprocal exchange in region A with conversion of *tk8* to wild type or by reciprocal exchange in region C with conversion of *tk26*. In either case, the proximal mutation is not converted, as discussed in the text. For orientation II direct repeats, the product of pathway 2 would be a $G418^s$ deletion product, which could be generated either through intrachromatid or through sister chromatid interactions. For orientation I direct repeats, the product of pathway 2 would be a triplication product for a sister chromatid crossover; the product of intrachromatid exchange would loop out and would not be recovered.

intrachromatid exchanges can occur (1, 27). Since we demonstrate that unequal sister chromatid interactions frequently produce triplication products, our results are consistent with models for gene amplification that propose a role for unequal sister chromatid recombination (e.g., reviewed in references 22 and 25). Moreover, unequal sister chromatid recombination in the germ line can lead to increases in gene copy number among members of multigene families (24, 29).

The observation that *tk* recombination in mouse L cells most frequently involves sister chromatid interactions suggests that the majority of reciprocal exchanges occur subsequent to DNA replication. In yeast cells, mitotic gene conversion is thought to occur predominantly in G_1, prior to DNA replication (4, 5), although reciprocal exchange may occur after DNA synthesis (17). The results of one yeast intrachromosomal recombination study that directly assessed the contributions of intrachromatid and sister chromatid crossovers show a predominance of intrachromatid interactions (9), in contrast to our contention for mouse L cells. A separate study in yeast cells showed a lower bias toward intrachromatid exchanges during intrachromosomal recombination (7).

Studies with yeast cells show that deletion and triplication products of direct repeats can be explained by a sister chromatid conversion mechanism (15, 18, 21). However, a sister chromatid conversion process does not readily explain our recovery of deletion products with the orientation II substrate. In addition, an explanation involving sister chromatid conversion in our substrates requires conversion of large heterologies (4.4 kb). Our studies indicate inefficient conversion of large heterologies (5a, 12). For these reasons, we favor reciprocal exchange, rather than conversion, as the mechanism responsible for generating the deletions and triplications seen in our study.

The relationship between homologous intrachromosomal recombination (in particular, that occurring between unequally paired sister chromatids as described in Fig. 1 to 3) and sister chromatid exchanges that are manifested cytologically (10) is unclear. In fact, agents that induce such sister chromatid exchanges (6, 10) do not necessarily enhance intrachromosomal homologous recombination in mammalian cells (6, 31). This lack of a correlation suggests to us that the two phenomena are not equivalent.

As indicated in Tables 2 and 3, deletion products arose at a low frequency during recombination between orientation II direct repeats. These recombinants are distinguished genetically as $G418^s$ HAT^r cells. In this study, we obtained 11 independent $G418^s$ HAT^r recombinants from orientation II direct-repeat lines. Results of G418 screening indicated that these arose at an overall frequency of 3% of all recombinants. This category (deletions of the *neo* gene) constituted 3%/20% (17% triplications + 3% deletions) or 15% of presumed reciprocal exchanges. However, such recombinants were not expected to occur through reciprocal exchange, as depicted in Fig. 3, since reciprocal exchange between orientation II direct repeats should lead to viable recombinants only in the case of sister chromatid crossovers (to generate triplications; Fig. 3). However, a reciprocal exchange accompanied by separated gene conversion can result in a TK^+ deletion product. For example, as depicted in Fig. 6, a viable recombinant could result if gene conversion at *tk8* is accompanied by reciprocal exchange in interval A. In turn, conversion of the *tk26* allele (proximal to the crossover site) does not occur in the same direction as at the *tk8* allele.

The mechanism in Fig. 6, elaborated to explain deletion recombinants in orientation II, would predict recovery of triplication recombinants in orientation I. Although one triplication recombinant was observed in this study, its molecular configuration was inconsistent with that predicted in Fig. 2 and 3 and hence is not included in Table 3. In our analysis of orientation II recombination, we screened for deletion products by assaying for G418 sensitivity, a more powerful assay than molecular hybridization analysis. Therefore, we were able to assay greater numbers of orientation II recombinants (our screen included 390 colonies, at least half of which arose independently) than of orientation I recombinants (we analyzed at the molecular level a total of 64 $G418^r$ colonies that could have represented triplication products). The lack of triplication products in orientation I may simply reflect this sampling bias.

Products of recombination involving conversion separated from reciprocal exchange have been observed in fungi. Explanations to account for such events invoke heteroduplex DNA as an intermediate (9, 16, 19, 20, 28, 32). Therefore, the observation of separated conversions observed in the orientation II analysis are consistent with the presence of heteroduplex DNA during intrachromosomal recombination in mammalian cells.

The primary goal of our study was to analyze the types of chromatid interactions between repeated genes. Because gene conversions were noninformative, products of reciprocal exchange between orientation I and orientation II direct repeats were compared. Whereas both sister chromatid and intrachromatid interactions could produce deletion products in orientation I, only sister chromatid interactions could give rise to triplication products in orientation II. Thus, the proportions of intrachromatid and sister chromatid interactions could be ascertained by dissecting the proportions of overall reciprocal exchanges in orientation I into two components: a sister chromatid component calculated from orientation II triplications, and an intrachromatid component that was inferred to be the remainder of that figure (reflecting intrachromatid crossovers that were not recovered in orientation II). On the basis of this analysis, we concluded that sister chromatid interactions predominate over intrachromatid interactions during direct-repeat recombination in mouse L cells. This predominance in turn implies that most reciprocal exchange occurs during or subsequent to DNA replication and provides a means for gene amplification via unequal sister chromatid recombination. Furthermore, observation of a class of recombinants produced by gene conversion separated from the site of associated reciprocal exchange implies the presence of heteroduplex DNA during intrachromosomal recombination.

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