*Proc. Natl. Acad. Sci. USA*
Vol. 84, pp. 5340–5344, August 1987
Genetics

# Differential effects of base-pair mismatch on intrachromosomal versus extrachromosomal recombination in mouse cells

**(homologous recombination/gene conversion/herpes simplex virus thymidine kinase gene/repeated sequences)**

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*Communicated by Franklin W. Stahl, March 27, 1987*

**ABSTRACT** **To initially determine the effect that base-pair mismatch has on homologous recombination in mammalian cells, we have studied genetic recombination between thymidine kinase (*tk*) gene sequences from herpes simplex virus 1 and 2. These *tk* genes are ≈81% homologous at the nucleotide level. We observed that, in mouse LTK$^-$ cells, intrachromosomal recombination between type 1 and type 2 *tk* sequences is reduced by a factor of at least 1000 relative to the rate of intrachromosomal recombination between homologous type 1 *tk* sequences. In sharp contrast, the rate of intermolecular or intramolecular extrachromosomal recombination between the heterologous *tk* sequences introduced by calcium phosphate or microinjection was reduced only by a factor of 3 to 15 compared with extrachromosomal homologous *tk* crosses. Our results suggest differences between the mechanisms of extrachromosomal and intrachromosomal recombination in mammalian cells.**

To elucidate the mechanism of homologous recombination in mammalian cells, investigators have studied genetic recombination using both extrachromosomal (1–10) and intrachromosomal (1, 11–14) systems. One important issue to address regarding homologous recombination is how the extent of homology between sequences influences the rate of recombination. Two groups have addressed this issue using extrachromosomal mammalian recombination systems (15, 16), whereas our laboratory has used an intrachromosomal system (17). The general finding is that when two sequences share several hundred base pairs (bp) of homology, the rate of recombination is proportional to the amount of homology. When the homology is reduced below ≈200 bp, the recombination rate drops off rapidly. In contrast to mammalian homologous recombination, prokaryotic recombination appears to require only ≈50 bp of homology to proceed efficiently (18–20).

In each of the above studies of the homology requirements of mammalian recombination, the rate of recombination was determined as a function of the extent of sequence overlap. Another way to study the homology requirements of recombination is to determine the effect that base-pair mismatch has on the recombination rate. It has been shown that the homologous recombination machinery of *Escherichia coli* is very sensitive to base-pair mismatch, with 16% mismatch resulting in a decrease by a factor of 100 in the rate of phage–plasmid recombination (19). In *E. coli*, the crucial factor that determines the recombination rate appears to be the length of *uninterrupted* stretches of homology (19, 20). In mammalian cells, it has been shown that recombination between the genomes of adenovirus types 2 and 5 can occur within regions exhibiting 90% sequence homology, but not within regions exhibiting 47% sequence homology (21).

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As a further step toward understanding the homology requirements of recombination in mammalian cells, we have studied genetic recombination between thymidine kinase (*tk*) gene sequences from herpes simplex virus 1 and 2 (HSV-1 and HSV-2, respectively). These *tk* genes are 81% homologous at the nucleotide level (22, 23). We have studied recombination between *tk* gene sequences using an intrachromosomal system and two extrachromosomal systems. The data presented in this report show that intrachromosomal recombination is reduced by more than a factor of 1000 by 19% base-pair mismatch, whereas extrachromosomal recombination is reduced by a factor of only 3–15.

## MATERIALS AND METHODS

**Cell Culture and Transfections.** Mouse L cells deficient in thymidine kinase (LTK$^-$ cells) were cultured and transfected by the calcium phosphate coprecipitate method (24), electroporation (25), or microinjection (26), as previously described.

**Determination of Extrachromosomal Recombination Rates.** Cells were transfected by the calcium phosphate coprecipitate method (24) or by direct microinjection (26) into the nucleus and were allowed to recover from the treatment for ≈20 hr in Dulbecco's modified Eagle's medium supplemented with 12% fetal calf serum (GIBCO). At this point, the cells were refed with media containing hypoxanthine/aminopterin/thymidine (HAT) (27). HAT-resistant colonies (HAT$^r$) were counted 14 days later, as a measure of the level of recombination to produce *tk*$^+$ cells that had occurred during the 20-hr period after transfection or microinjection.

**Determination of Intrachromosomal Recombination Rates.** Fluctuation analysis tests were done as described (28).

**Plasmid Constructions.** The vector used in all constructions is a derivative of pSV2-neo (30) with restriction site alterations as described (28). *Xho* I linker insertion mutations of the HSV-1 (strain F) thymidine kinase (*tk*) gene were gifts from D. Zipser and J. Kwoh (Cold Spring Harbor Laboratory). The HSV-2 (strain 333) *tk* gene was a gift from D. Galloway. Mutant no. 8 contains an 8-bp *Xho* I linker inserted at nucleotide 1215 of the HSV-1 *tk* gene, whereas mutant no. 28 contains an 8-bp *Xho* I linker inserted at nucleotide 1036 of the HSV-1 *tk* gene [numbering according to Wagner *et al.* (31)]. Plasmid pTK1-8 contains the no. 8 mutant *tk* gene inserted on a 2.4-kb *Bam*HI fragment into the unique *Bam*HI site of the pSV2-neo vector after attachment of *Bam*HI linkers (New England Biolabs); plasmid pTK1-28 is a similar construct containing the no. 28 mutant *tk* gene on a 2.4-kb *Bam*HI fragment.

The 800-bp *Eco*RV–*Stu* I restriction fragment of the HSV-2 *tk* gene was isolated. This fragment is missing the 30% of the

Abbreviations: HSV-1, herpes simplex virus 1; HSV-2, herpes simplex virus 2; HAT medium, hypoxanthine/aminopterin/thymidine-containing medium that selects against *tk*$^-$ cells; HAT$^r$, HAT-resistant colonies (*tk*$^+$).

coding region of the HSV-2 *tk* gene that maps upstream from the *Eco*RV site and does not contain the polyadenylylation signals of the HSV-2 *tk* gene that map downstream from the *Stu* I site (22, 23). Using *Hin*dIII linkers (New England Biolabs), this fragment was then inserted into the unique *Hin*dIII sites of the pSV2-neo vector, pTK1-8, and pTK1-28 to produce, respectively, plasmids pTK2, pTK2TK1-8, and pTK2TK1-28.

Plasmid pAL5 is identical to pTK2TK1-8 except that it contains the 1.2-kb *Hinc*II–*Sma* I fragment of the HSV-1 (strain F) *tk* gene inserted at the *Hin*dIII site, using *Hin*dIII linkers. The fragment encodes most of the coding region but lacks the *tk* gene promoter and polyadenylylation signals (31). Plasmid pAL2 contains the 1.2-kb *Hinc*II–*Sma* I fragment of the HSV-1 *tk* gene inserted into the *Hin*dIII site of the pSV2-neo vector.

**Southern Hybridization Analysis.** DNA isolation and Southern blotting analysis were accomplished as described (28).

## RESULTS

**Intrachromosomal Recombination Between HSV-1 and HSV-2 *tk* Sequences.** We have studied intrachromosomal recombination between *tk* gene sequences from HSV-1 and HSV-2. These genes are 81% homologous at the nucleotide level, whereas the proteins are 75% homologous (22, 23). The nucleotide mismatches between the genes are fairly evenly distributed, with the longest stretches of perfect sequence match being ≤30 bp. Two plasmids, pTK2TK1-8 and pTK2TK1-28, containing two different *Xho* I linker insertion mutations of the HSV-1 (strain F) *tk* gene were constructed. These plasmids also contain a defective fragment of the wild-type *tk* gene from HSV-2 (strain 333) as well as the neomycin resistance gene. Maps of these plasmids are shown in Fig. 1. Both plasmids were linearized by digestion with restriction endonuclease *Cla* I and then transfected into mouse LTK$^-$ cells. Cell lines stably resistant to G418 and containing one or several copies of pTK2TK1-8 or pTK2TK1-28 integrated into the L cell genome were isolated. The rate of intrachromosomal recombination between the wild-type fragment of the HSV-2 *tk* gene and the no. 8 or no. 28 *Xho* I linker insertion mutant HSV-1 *tk* gene was determined by fluctuation tests, using HAT selection to monitor *tk*$^+$ segregants. Table 1 shows that we were unable to recover any recombinants in any of the five cell lines containing pTK2TK1-8 or pTK2TK1-28. The rate of recovery of *tk*$^+$ recombinants between the heterologous *tk* sequences was determined to be $<10^{-9}$ events per locus per cell generation. In comparison, the rate of intrachromosomal recombination between a defective internal fragment of the HSV-1 *tk* gene and the no. 8 mutant HSV-1 *tk* gene in cell lines containing pAL5 (Fig. 1) is $\approx 10^{-6}$ events per locus per cell generation (17). [Intrachromosomal recombination between the no. 28 mutant HSV-1 *tk* gene and HSV-1 sequences also occurs at a rate of $\approx 10^{-6}$ (28).] Constructs pAL5 and pTK2TK1-8 differ in two respects; the wild-type *tk* sequence on pAL5 is 1.2 kb in length and is perfectly homologous to the mutant no. 8 gene (excluding the *Xho* linker insertion mutation), whereas the wild-type (type 2) *tk* sequence on pTK2TK1-8 is 800 bp in length and exhibits 19% base-pair mismatch with the mutant no. 8 gene. From previous work (17), we know that the difference between 800 bp and 1.2 kb of sequence overlap cannot account for a 1000-fold difference in the rate of recombination. Therefore, the ≥1000-fold change in the rate of recovery of recombinants seen with pTK2TK1-8 compared with pAL5 was due to the 19% base-pair mismatch between the HSV-1 and HSV-2 *tk* genes.

**Extrachromosomal Recombination Between Sequences Introduced into Cells by Calcium Phosphate Coprecipitation.**

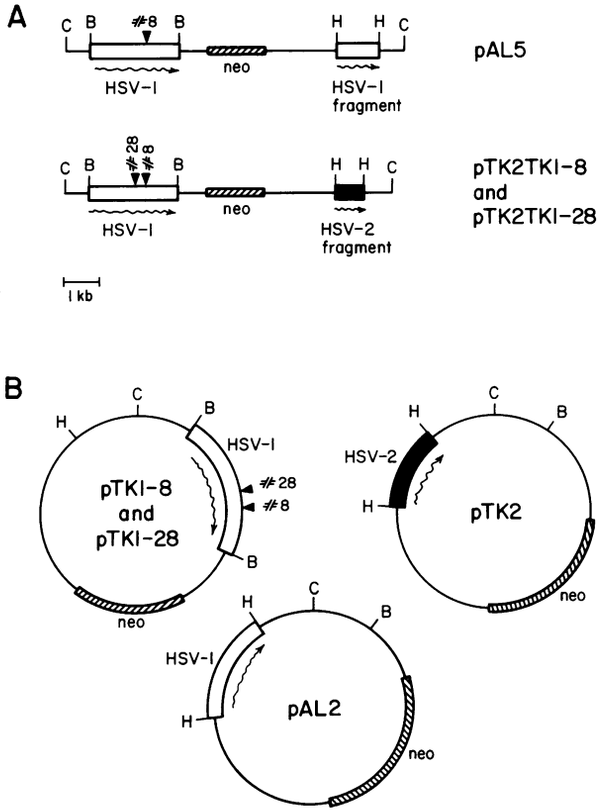


FIG. 1. Maps of recombination substrates. *Bam*HI (B), *Cla* I (C), and *Hin*dIII (H) sites are indicated, as well as the positions of *Xho* I linker insertion mutations 8 and 28. Wavy arrows indicate the 5′ to 3′ orientation of the *tk* gene sequences. ▭, ■, ▨, and — represent HSV-1, HSV-2, neomycin resistance gene, and vector sequences, respectively. (*A*) Constructs pAL5 and pTK2TK1-8 (or/and pTK2TK1-28) linearized at the *Cla* I site. This is the configuration of the *tk* gene sequences when integrated into the L cell genomes in the intrachromosomal recombination studies. (*B*) Constructs pTK1-8 (8.3 kb), pTK1-28 (8.3 kb), pTK2 (6.6 kb), and pAL2 (7.0 kb).

Our inability to recover HSV-1 × HSV-2 recombinants in the intrachromosomal studies presented above was due to a lack of actual recombination between the heterologous genes and/or the formation of nonfunctional hybrid HSV-1/HSV-2 *tk* genes as products of recombination. Because the rate of

Table 1. Effect of 19% base-pair mismatch on the rate of intrachromosomal recombination

| Cell line | Copy no. | Cells tested, no. | Recombinants, no. | Rate of recombination |
|---|---|---|---|---|
| 8–1 | 1 | $2.0 \times 10^8$ | 0 | $<1 \times 10^{-9}$ |
| 8–2 | 1 | $4.6 \times 10^8$ | 0 | $<1 \times 10^{-9}$ |
| 8–3 | 1 | $4.0 \times 10^8$ | 0 | $<1 \times 10^{-9}$ |
| 28–1 | 1 | $4.0 \times 10^8$ | 0 | $<1 \times 10^{-9}$ |
| 28–2 | 3 | $4.0 \times 10^8$ | 0 | $<1 \times 10^{-9}$ |

Cell lines designated 8-1, -2, and -3 contain the construct pTK2TK1-8 stably integrated into their genomes, whereas cell lines designated 28-1 and 28-2 contain the construct pTK2TK1-28 integrated into their genomes. Rates of recombination (events per generation) between the HSV-1 and HSV-2 *tk* gene sequences in these cell lines were estimated from the observed recombination frequencies as described. For comparison, the average rate of recombination for four lines containing pAL5 (in which the interacting *tk* sequences are perfectly homologous) was previously determined (17) to be $0.8 \times 10^{-6}$, at least 1000-fold greater than the rate of recombination between *tk* sequences exhibiting 19% base-pair mismatch.

extrachromosomal recombination is, in general, much higher than intrachromosomal recombination (1, 4, 6–9, 32), we examined extrachromosomal recombination of HSV-1 and HSV-2 *tk* gene sequences to obtain some evidence that hybrid *tk* proteins are, indeed, functional.

Plasmid pTK2TK1-8 or pAL5 was introduced into mouse LTK$^-$ cells by the calcium phosphate coprecipitate method (26). As shown in Table 2, cells transfected with uncut pAL5 (containing homologous *tk* genes) gave rise to three times as many HAT colonies as did cells transfected with uncut pTK2TK1-8 (containing heterologous *tk* genes). The number of HAT$^r$ colonies was proportional to the amount of plasmid DNA transfected, over a 10-fold range of DNA concentration, for both pAL5 and pTK2TK1-8 (data not shown). This indicated that the amount of plasmid DNA used was not saturating and that counting colonies therefore provided a valid determination of the relative recombination rates for the homologous and heterologous *tk* gene crosses. When pAL5 and pTK2TK1-8 were linearized by digestion with *Xho* I before transfection, the number of recombinants increased ≈10-fold for both constructs (Table 2). Such stimulation of extrachromosomal recombination by a double-strand break in a region of shared homology has been observed in several previous studies (4, 5, 12, 33–35).

As shown in Table 2, cotransfection of two type 1 *tk* sequences on separate plasmids (pAL2 and pTK1-8, Fig. 1) yielded recombinants at a 15-fold greater rate than that observed after cotransfection of the heterologous type 1 and type 2 *tk* sequences (pTK2 and pTK1-8, Fig. 1). When the plasmid harboring the no. 8 mutant HSV-1 *tk* gene (pTK1-8) was linearized by digestion with *Xho* I before cotransfection with an homologous (pAL2) or a heterologous (pTK2) partner, the rate of recombination was increased 10-fold or 20-fold, respectively. The rate of such intermolecular recombination exhibited a linear dependence on the amount of *Xho* I-cleaved pTK1-8 DNA transfected into the cells, indicating again that the number of HAT$^r$ colonies was not saturated under the conditions employed (data not shown).

A second type 1 mutant gene, no. 28, was also linearized by digestion with *Xho* I and cotransfected into L cells with pAL2 or pTK2. The recombination rate observed with this second homologous (1 × 1) cross was 10-fold greater than the rate of recombination of the corresponding heterologous (1 × 2) cross (Table 2).

Table 2. Effect of 19% base-pair mismatch on the rate of extrachromosomal recombination after calcium phosphate transfection

| Plasmid(s) | Dishes, total no. | Colonies per dish | Ratio, (1 × 1):(1 × 2) |
|---|---|---|---|
| Intramolecular recombination | | | |
| pAL5 | 18 (3)* | 9 | 3:1 |
| pTK2TK1-8 | 23 (3) | 3 | |
| pAL5/*Xho* I | 5 (2) | 117 | 3:1 |
| pTK2TK1-8/*Xho* I | 13 (2) | 36 | |
| Intermolecular recombination | | | |
| pAL2 + pTK1-8 | 9 (3) | 8 | 15:1 |
| pTK2 + pTK1-8 | 23 (3) | 0.52 | |
| pAL2 + pTK1-8/*Xho* I | 8 (3) | 112 | 8:1 |
| pTK2 + pTK1-8/*Xho* I | 15 (3) | 14 | |
| pAL2 + pTK1-28/*Xho* I | 8 (3) | 126 | 10:1 |
| pTK2 + pTK1-28/*Xho* I | 15 (3) | 13 | |
| Controls | | | |
| wt HSV-1 *tk* gene | 20 (10) | ≈1000 | |
| pTK2 | 5 (1) | 0 | |
| pTK1-8 + pTK1-8/*Xho* I | 5 (1) | 0 | |

LTK$^-$ cells, 5 × $10^5$ per dish, were transfected with 1 μg of the indicated construct plus 14 μg of LTK$^-$ DNA, or, in the case of cotransfection experiments, cells were transfected with 10 μg of each construct plus no carrier LTK$^-$ DNA.

*Numbers in parentheses indicate the number of independent experiments that were done for each case.

**Extrachromosomal Recombination Between Sequences Introduced into Cells by Microinjection.** Plasmids pAL5 and pTK2TK1-8 were linearized by digestion with *Xho* I and microinjected directly into the nuclei of LTK$^-$ cells, at ≈20 copies per cell. HAT$^r$ colonies arose at a 15-fold greater rate for cells injected with the homologous substrate, pAL5, as compared with cells injected with the heterologous substrate pTK2TK1-8 (Table 3). We estimate that ≈10%–20% of the cells receiving pAL5/*Xho* I had undergone a recombination event.

**Analysis of Recombinant HSV-1 × HSV-2 *tk* Genes Produced by Extrachromosomal Recombination.** To examine the *tk* genes present in the HAT$^r$ colonies produced by extrachromosomal recombination, DNA isolated from several such cell lines was analyzed by Southern blotting (Fig. 2). Out of 17 recombinants examined, 7 were consistent with a gene conversion (or a double exchange) event in which the defective HSV-2 *tk* gene sequence donated wild-type information to the mutant HSV-1 *tk* gene, thus eliminating the *Xho* I site and producing a 2.4-kb *Bam*HI fragment resistant to *Xho* I digestion (see Fig. 2).

Ten recombinants displayed a 1.9-kb fragment upon digestion with *Bam*HI and *Hin*dIII (see Fig. 2), consistent with a single crossover between the HSV-1 and HSV-2 *tk* sequences in which the 5′ portion of the recombinant gene is composed of HSV-1 sequences and the 3′ portion of the recombinant gene is composed of HSV-2 sequences.

Several [(mutant no. 8) × HSV-2 and (mutant no. 28) × HSV-2] recombinants were examined in greater detail. For some (two of four) of the recombinants analyzed that had apparently undergone a gene conversion, further restriction analysis revealed that these *tk* genes were clearly hybrid genes, each containing a segment of the HSV-2 *tk* sequence replacing HSV-1 sequence in a domain encompassing the position of the insertion mutations. The amount of sequence information that had been transferred was between 300 and 800 bp (Fig. 3*C*). In contrast, the other recombinants that apparently arose from gene conversion did not contain any HSV-2 *tk* restriction sites, indicating that only a small amount (<100 bp) of sequence was transferred in these cases (Fig. 3*D*).

## DISCUSSION

An important question to address regarding homologous recombination is precisely how well-matched two sequences must be in order to undergo recombination. There have been several investigations into the homology requirements of recombination in mammalian cells (15–17). These studies, however, all utilized essentially perfectly homologous substrates sharing varying lengths of overlapping homology. We have begun to address the question of how recombination in mammalian cells is affected by varying degrees of base-pair mismatch. We find that the 19% base-pair mismatch that

Table 3. Effect of 19% base-pair mismatch on the rate of extrachromosomal recombination of microinjected substrates

| Construct injected | Copies injected per cell | Cells injected, no. | HAT$^r$ colonies scored, no. | Relative recombination rate* |
|---|---|---|---|---|
| Wild-type *tk* gene | 10 | 446 | 36 | — |
| pAL5/*Xho* I | 20 | 2500 | 38 | 15 |
| pTK2TK1-8/*Xho* I | 20 | 3020 | 3 | 1 |

*Relative rate is normalized to the frequency of recombinants following injection of pTK2TK1-8/*Xho* I, which is ≈1 per 1000 cells injected.

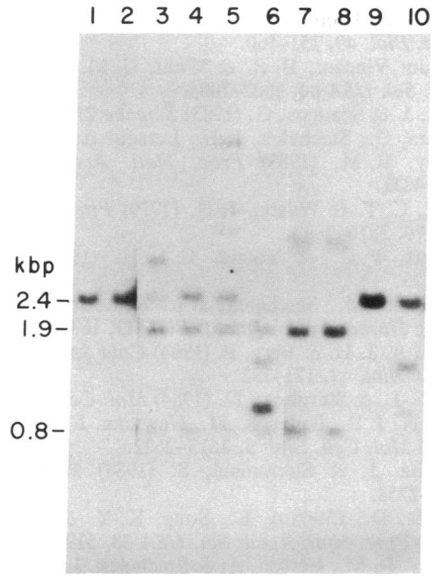


FIG. 2. Southern blotting analysis of representative *tk* genes produced by extrachromosomal recombination. DNA was analyzed using a probe specific for HSV-1 and HSV-2 *tk* sequences. Shown is the analysis of DNA from five recombinants that arose from extrachromosomal recombination between HSV-2 *tk* sequences and the no. 8 mutant HSV-1 *tk* gene, following calcium phosphate-mediated transfection. Pairwise lanes (e.g., lanes 1 and 2) represent individual recombinants digested with *Bam*HI and *Hin*dIII (odd-numbered lanes) or *Bam*HI, *Hin*dIII, and *Xho* I (even-numbered lanes). Indicated are the mobilities of the 2.4-kb fragment diagnostic for gene conversions and the 1.9-kb fragment diagnostic for single crossovers. Also indicated is the mobility of the 800-bp *Hin*dIII insert (containing HSV-2 *tk* sequences) present in the constructs used. Every line shown appears to contain a reconstructed *tk* gene. The samples displayed in lanes 1,2 and 9,10 each arose from a gene conversion; the samples in lanes 5,6 and 7,8 each arose from a single crossover; the sample in lanes 3,4 had either undergone both a gene conversion and a crossover event or fortuitously exhibits a fragment of 2.4 or 1.9 kb. A similar array of recombinants arose from extrachromosomal recombination between HSV-2 *tk* sequences and the no. 28 mutant HSV-1 *tk* gene (data not shown).

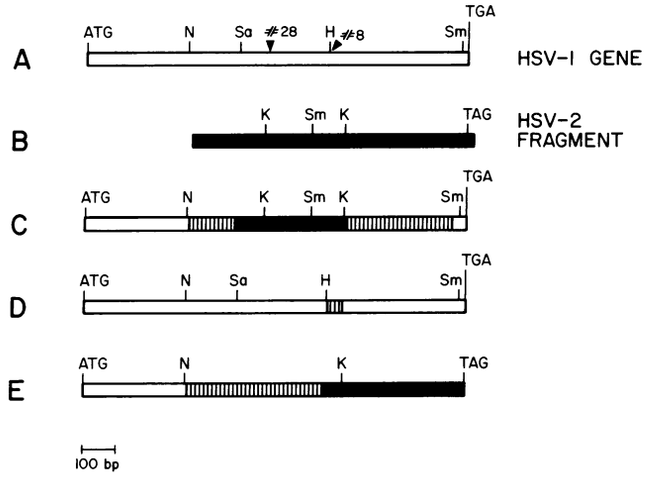


FIG. 3. Structures of recombinant *tk* genes produced by extrachromosomal recombination. After initial characterization by digestion with *Bam*HI, *Hin*dIII, and *Xho* I, as illustrated in Fig. 2, several recombinant *tk* genes were further analyzed by digestion with *Hin*fI (H), *Kpn* I (K), *Nru* I (N), *Sac* I (Sa), and *Sma* I (Sm). Shown are schematic representations of the recombinant gene structures determined. □ and ■ represent HSV-1 and HSV-2 *tk* gene sequences, respectively. ▥ represents sequence that could not be unambiguously assigned as type 1 or type 2 but that necessarily contains the junction between type 1 and type 2 sequence in the recombinant genes. (*A*) HSV-1 *tk* gene showing the locations of *Xho* I linker insertion mutations no. 8 and no. 28. (*B*) HSV-2 *tk* gene fragment used in these studies. (*C*) Recombinant genes arising from apparent gene conversions that corrected the no. 8 or no. 28 mutant. Sequence information of 300–800 bp was transferred from the type 2 sequence to the mutant type 1 gene in this type of event, replacing a portion of the type 1 gene that had contained the no. 8 or no. 28 mutation. (*D*) Recombinant gene resulting from correction of the no. 8 HSV-1 mutant gene by a short conversion tract (<60 bp, the distance from the *Hin*fI site in HSV-1 *tk* to the downstream *Kpn* I site in HSV-2 *tk*). This gene does not contain HSV-2 restriction sites for any restriction enzyme tested. Similar recombinant genes in which the no. 28 mutant gene was corrected by a short (<100 bp) conversion tract were observed. (*E*) Family of recombinant genes arising from single crossovers between the HSV-2 sequence and the no. 8 mutant HSV-1 gene. A similar family of genes was produced by single crossovers between the HSV-2 sequence and the no. 28 mutant HSV-1 gene.

exists between the HSV-1 and the HSV-2 *tk* genes can reduce intrachromosomal recombination by a factor of at least 1000 relative to the rate of intrachromosomal recombination observed for two HSV-1 *tk* gene sequences. This result was obtained using "crosses" of the same HSV-2 *tk* sequence with two different *Xho* I linker insertion mutants of the HSV-1 *tk* gene, indicating that this sensitivity to mismatch is not specific to some particular region of the *tk* gene.

Using the same recombination substrates as were used in our intrachromosomal studies, we found that extrachromosomal recombination is considerably less sensitive to base-pair mismatch. When the HSV-1 and HSV-2 *tk* sequences were introduced into L cells on the same DNA molecule or on separate molecules, by calcium phosphate coprecipitation, the rate of extrachromosomal recombination was only reduced by a factor of 3–15 compared with the extrachromosomal recombination rate of comparable homologous *tk* crosses. The relative rates of recombination of the homologous versus heterologous *tk* crosses were not affected by linearization at the site of the mutation in the HSV-1 *tk* gene.

Introduction of DNA into mammalian cells by calcium phosphate transfection could result in damage or processing of DNA constructs during passage through the cytoplasm, which might have an effect on recombination. We therefore performed experiments in which the constructs were introduced by direct microinjection into the nucleus. We found that the relative rate of extrachromosomal recombination of the heterologous *tk* cross compared with the homologous *tk* cross was the same (that is, reduced by a factor of ≈15) as in the calcium phosphate coprecipitation experiments. This strongly suggests that this relative insensitivity to base-pair mismatch is a feature of extrachromosomal recombination *per se*.

Molecular analysis of several extrachromosomal recombinants indicated that a variety of functional hybrid HSV-1/HSV-2 *tk* genes were produced that conferred HAT$^r$ when present as a single copy gene in L cells. This substantiated that the failure to recover HSV-1 × HSV-2 *tk* recombinants intrachromosomally was not solely the result of faulty hybrid *tk* proteins, but rather was the reflection of a reduced rate of recombination between the heterologous sequences.

The observed difference in sensitivity to base-pair mismatch displayed by intrachromosomal and extrachromosomal recombination in mammalian cells has several possible explanations. Perhaps recombination of chromosomal versus extrachromosomal sequences is accomplished by two distinct pathways, requiring different (perhaps overlapping) sets of gene products. In bacteria, it is well documented that genetic blocks differentially affect recombination, depending on the parental configurations of DNA (36, 37). Phage–episomal F-factor recombination is almost completely blocked by *recB*$^-$ mutations, whereas F-factor–chromosome crosses are virtually unaffected (36). A second possibility is

that the *nature* of the substrates exerts an influence on the homology requirements of mammalian recombination. The intrachromosomal substrates are presumably coated with histones and assembled into chromatin, whereas the extrachromosomal substrates presumably exist as naked DNA molecules when introduced into the cells. Another difference between the substrates is that there could be more available free ends of DNA for extrachromosomal recombination than for intrachromosomal recombination. It is possible that such intrinsic characteristics of the recombination substrates, and not their locations *per se* (chromosomal versus extrachromosomal), determine which recombination pathway will be used. Alternatively, a third possibility is that a single recombination pathway (i.e., a single set of gene products) operates on both intra- and extrachromosomal substrates in mammalian cells, but the intrinsic characteristics of each substrate may influence the manner in which the substrate interacts with the recombination machinery, and this, in turn, may affect the homology requirements.

Although it is not presently clear why intrachromosomal and extrachromosomal recombination have such different homology requirements, this difference should be kept in mind when extrapolating results of extrachromosomal recombination studies to learn about intrachromosomal events, and vice versa. The possibility of mechanistic differences between intra- and extrachromosomal recombination may also prove relevant in developing strategies for manipulating the genome by targeted recombination because, in a certain sense, targeting involves both intra- and extrachromosomal recombination.

A stringent homology requirement for chromosomal recombination as deduced from the studies presented here would be sufficient to prevent frequent "unwanted" recombination between abundant repeated sequences such as the *Alu* and *Kpn* I repeats, the family members of which contain sequences that exhibit about 80–85% homology (29, 38). Finally, whether the rate of intrachromosomal recombination is determined primarily by the overall *degree* of heterology or by the *distribution* of mismatches can be addressed by using gene sequences that are more closely matched than those used in this study.

The authors thank Dr. Hamish Young, Dr. Kevin Kelley, Dr. Barbara Criscuolo Waldman, Roni Bollag, and Alan Godwin for their valuable comments. We also thank Janet Stachelek for her excellent technical assistance. This work was supported by National Institutes of Health Grant GM32741 to R.M.L. and P01 CA39238 to Dr. William C. Summers. R.M.L. is a Leukemia Society of America Scholar, and A.S.W. is a Leukemia Society of America Fellow.

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