MOLECULAR AND CELLULAR BIOLOGY, Apr. 1992, p. 1546–1552
0270-7306/92/041546-07$02.00/0

Vol. 12, No. 4

Formation of Heteroduplex DNA during Mammalian Intrachromosomal Gene Conversion

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Received 17 October 1991/Accepted 8 January 1992

We have studied intrachromosomal gene conversion in mouse *Ltk*$^-$ cells with a substrate designed to provide genetic evidence for heteroduplex DNA. Our recombination substrate consists of two defective chicken thymidine kinase genes arranged so as to favor the selection of gene conversion products. The gene intended to serve as the recipient in gene conversion differs from the donor sequence by virtue of a palindromic insertion that creates silent restriction site polymorphisms between the two genes. While selection for gene conversion at a *Xho*I linker insertion within the recipient gene results in coconversion of the nearby palindromic site in more than half of the convertants, 4% of convertant colonies show both parental and nonparental genotypes at the polymorphic site. We consider these mixed colonies to be the result of genotypic sectoring and interpret this sectoring to be a consequence of unrepaired heteroduplex DNA at the polymorphic palindromic site. DNA replication through the heteroduplex recombination intermediate generates genetically distinct daughter cells that comprise a single colony. We believe that the data provide the first compelling genetic evidence for the presence of heteroduplex DNA during chromosomal gene conversion in mammalian cells.

When uncorrected mispairs arise in duplex DNA, semiconservative DNA replication of the resultant heteroduplex segregates genetically distinct daughter molecules. Since one source for the generation of heteroduplex DNA is the process of genetic recombination, the two strands of a single DNA molecule can emerge from meiosis as nonidentical daughter molecules. Whereas four pairs of genes synapse during meiosis (4:4), fungal meiosis occasionally produces uneven aberrant segregations such as 5:3 or 3:5. Such postmeiotic segregations (PMS) are thought to result from the mitotic replication of heteroduplex DNA produced during genetic recombination.

Most current models for genetic recombination derive from a model proposed by Holliday (15), who interpreted PMS as nonrepair of heteroduplex DNA (hDNA) and suggested that gene conversion resulted from correction of mismatches in hDNA. Although current models for meiotic recombination invoke different initiation mechanisms for gene conversion, such as single-strand invasion (22) or double-strand gap formation (30), these models generally suggest that heteroduplex formation may accompany gene conversion. In fungi, the most compelling genetic evidence for hDNA is the observation of PMS during meiotic recombination (16, 26) or of sectored colonies during mitotic recombination (8, 11, 28, 36). Such recombinants most likely result from the failure to repair hDNA intermediates prior to DNA replication.

Evidence for the existence of heteroduplex DNA in mammalian cells is primarily indirect. Results of both in vivo and in vitro studies indicate that mammalian cells do possess the ability to process preformed mismatched DNA, hDNA, to the homoduplex forms following transfection (1, 2, 6, 9, 10, 13, 32, 33). Correlative studies with Chinese hamster cells showed that the addition of DNA-damaging agents, known to increase recombination frequencies in some organisms, increased the appearance of hybrid DNA, as measured by a physical assay (23, 27). A novel type of induced mutagenesis, observed during gene targeting studies in mouse cells, appeared to be facilitated by heteroduplex DNA formation (31). In addition, studies with mouse L cells have provided indirect evidence that hDNA has a role in mammalian intrachromosomal gene conversion (5, 19). However, direct evidence for hDNA formation during chromosomal gene conversion in mammalian cells is lacking.

As discussed above, the primary evidence for hDNA in fungal gene conversion is the observation of PMS resulting from meiotic recombination. Since the degree of PMS in fungi is highly dependent on the particular marker involved, specific mismatches in DNA are thought to be corrected to different degrees by the mismatch repair machinery (3, 34, 35). Recently, Nag et al. (24, 25) have shown that palindromic insertions appear to elude the mismatch repair machinery and thereby generate high levels of PMS.

With fungal recombination as a paradigm, we have used a sectored (mixed) colony assay to detect the formation of hDNA during intrachromosomal gene conversion in cultured mouse cells. As schematized in Fig. 1, the system utilizes a recombination substrate containing a direct repeat of a selectable marker, the chicken thymidine kinase (*tk*) gene. The defective donor and recipient sequences of the substrate manifest a silent polymorphism (a palindromic insertion in the recipient). During gene conversion, formation of hDNA at this polymorphic site without correction prior to DNA replication would produce daughter cells that differ with respect to the presence of the polymorphism. Subsequent coherent cell division of the two daughter cells is expected to lead to sectored colonies that can be detected by molecular analysis. Using this system, we have observed sectored recombinant colonies that provide the first compelling evidence for the formation of hDNA during chromosomal gene conversion in a mammalian system.

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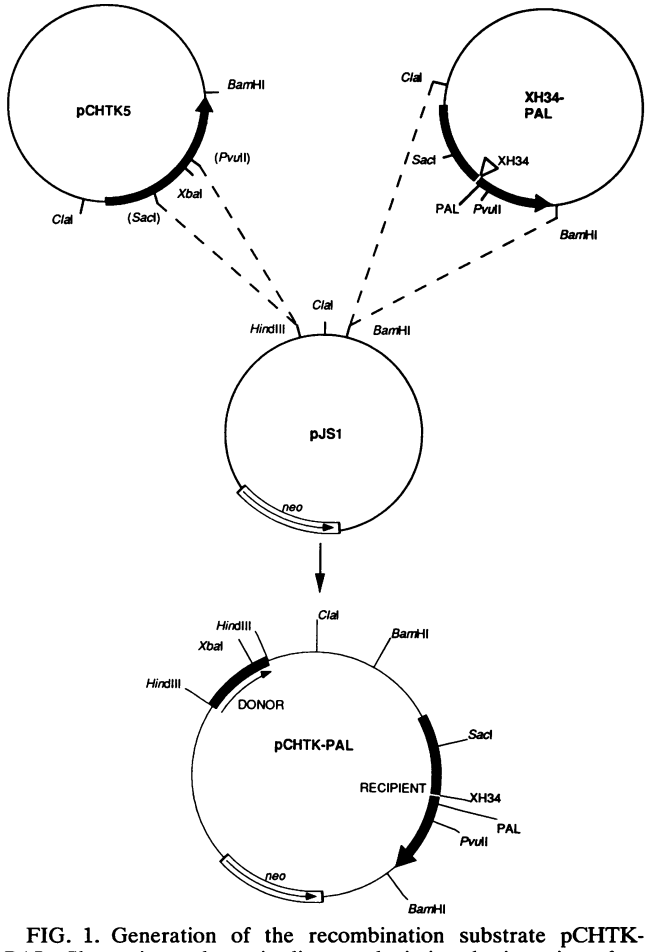

FIG. 1. Generation of the recombination substrate pCHTK-PAL. Shown is a schematic diagram depicting the insertion of an internal donor fragment of pCHTK5 into the *Hin*dIII site of pJS1 and of a modified XH34-PAL full-length mutant recipient chicken *tk* gene into the *Bam*HI site. Both the donor and recipient genes are transcribed in the same direction (clockwise in pJS1 as depicted). Greater detail of the polymorphisms between donor and recipient genes is presented in Fig. 2.

MATERIALS AND METHODS

Plasmids. Plasmid pCHTK-PAL (Fig. 1) was created from pJS1, a derivative of pSV2*neo* described previously (21). A 922-bp donor fragment and a 3,105-bp recipient fragment were derived from the chicken *tk* gene (17). The internal fragment was generated by cleaving pCHTK5 with *Sac*I, filling in the *Sac*I overhang with T4 DNA polymerase, and then cleaving with *Pvu*II. The fragments were then ligated to kinase-treated *Hin*dIII linkers, and the 922-bp fragment was isolated and ligated into the *Hin*dIII site of pJS1. The recipient gene was created by cleaving the derivative of pCHTK5 containing a *Xho*I linker at position 1932 (XH34) (17, 18) with *Xba*I. A 34-bp palindromic oligonucleotide was prepared by denaturing and slow reannealing of a 34-bp oligonucleotide (prepared by Monica Talmor, Yale University) of the sequence CTAGTCGATATCGCCAGGGCCC TGGCGATATCGA. This fragment containing *Xba*I-compatible ends (but eliminating the *Xba*I site) was inserted into the last intron by ligation into *Xba*I-cut XH34. The resulting plasmid was then cleaved with *Cla*I and ligated to *Bam*HI linkers, and a 3,105-bp *Bam*HI fragment containing the recipient gene was isolated and inserted into the *Bam*HI site of pJS1 containing the donor gene. Double-stranded DNA sequencing was performed with the Sequenase kit (U.S. Biochemicals), using protocols supplied by the manufacturer.

Generation of cell lines. Cell lines were generated by direct nuclear microinjection of *Cla*I-linearized pCHTK-PAL (0.5 μg/ml) into Ltk^- cells (7). Transformants were selected in medium containing 400 μg of G418 sulfate (Geneticin; GIBCO) per ml. Two single-copy cell lines, PAL-1 and PAL-4, identified by a strategy outlined previously (19), were chosen for recombination analysis.

Isolation of recombinants. To score only newly arising conversion products, preexisting recombinants were eliminated by selection of parental lines in medium containing 5 μg of trifluorothymidine (TFT) per ml for 8 to 12 days. Cells were then trypsinized and plated into 6-well (PAL-4) or 24-well (PAL-1) cell chambers containing 150 μM thymidine (excess thymidine to dilute remaining TFT) at a density of 100,000 or 20,000 cells, respectively. After 2 days, the medium was replaced with HAT_{10} (100 μM hypoxanthine, 2 μM aminopterin, 150 μM thymidine) to select thymidine kinase prototrophs that had arisen in the preceding 2 days. Chambers were then scored after ~12 days for wells that contained single colonies; these colonies were isolated and grown for DNA analysis.

Molecular analysis of recombinants. Techniques for the preparation of genomic DNA and Southern blot hybridization analysis have been described previously (4). Polymerase chain reaction (PCR) amplifications (29) were performed in 100-μl reaction volumes containing 1 μg of genomic DNA in a mixture of 50 mM KCl, 10 mM Tris-Cl (pH 9.0), 1.5 mM $MgCl_2$, 0.01% gelatin, primers at 0.1 μM, deoxynucleoside triphosphates at 200 μM, and 2.5 U of *Taq* polymerase (Cetus). Reactions were carried out in a Perkin-Elmer/Cetus DNA Thermal Cycler as follows: 1 min at 94°C, 1 min at 65°C, and 1.5 min at 72°C for 35 cycles. Primers were specific for the recipient gene (bp 1248 to 1267 and bp 2335 to 2316 in reference 17). Amplified sequences corresponding to the recipient gene(s) present in genomic DNAs from single colonies were then cleaved with restriction enzymes specific for sites in the donor gene (*Xba*I) or in the recipient gene (*Eco*RV and *Bst*XI). Sectoring was diagnosed by a mixed pattern of cleavage with two or three of the diagnostic enzymes. To ensure complete digestion, restriction enzyme digestions were performed with greater than fivefold excess of enzymes.

RESULTS

Rationale. We have designed an assay to detect unrepaired heteroduplex DNA accompanying gene conversion in mammalian cells. The assay involves an intrachromosomal recombination substrate that consists of a pair of linked chicken *tk* genes, one full length and the other truncated at its 5′ and 3′ ends. This substrate is intended to restrict recovery of recombinants to those consistent with gene conversion. Within the substrate, the sequence intended to act as the recipient in gene conversion is differentiated from the donor by an 8-bp *Xho*I linker (XH34) inserted into the sixth (penultimate) exon, 93 bp upstream from a 34-bp perfect palindrome inserted into the last intron of the chicken *tk* gene (Fig. 2). Colonies selected for gene conversion at XH34 are examined with respect to nearby restriction site polymorphisms comprising the palindrome insertion. Colonies with a mixed pattern of cleavage are interpreted as

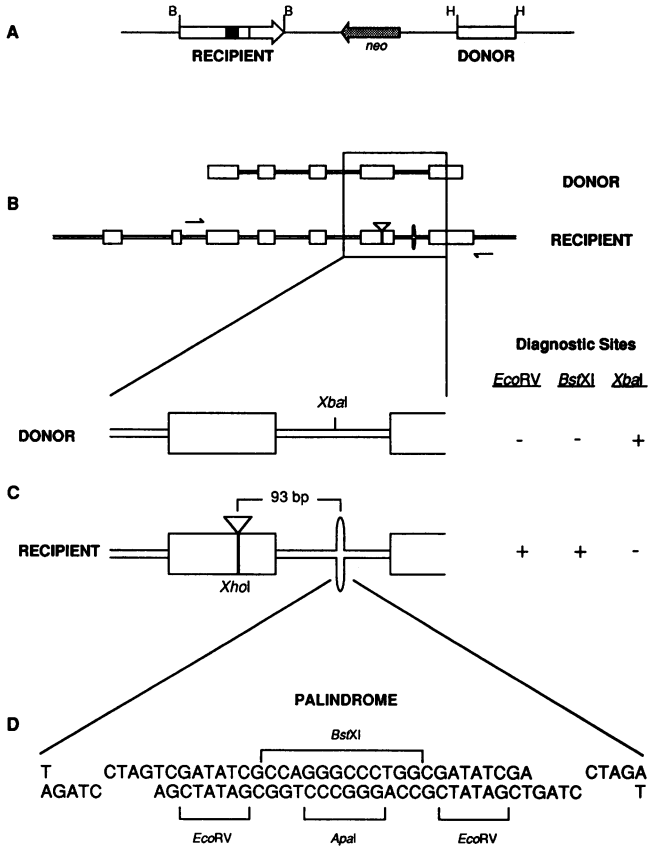

FIG. 2. Comparison of the recipient and donor genes in pCHTK-PAL. (A) Configuration of recombination substrate integrated into the parent line. Mutation XH34 (filled box) and the palindrome (line) are indicated in the recipient gene. (B) Intron/exon structures of the donor and recipient genes. Arrows indicate the positions of primers used in PCR amplification of the recipient gene. (C) Detail of the last intron in both the donor and recipient sequences, with the diagnostic restriction sites indicated. (D) Sequence of the palindrome insertion within the last intron of the recipient gene, with restriction sites indicated.

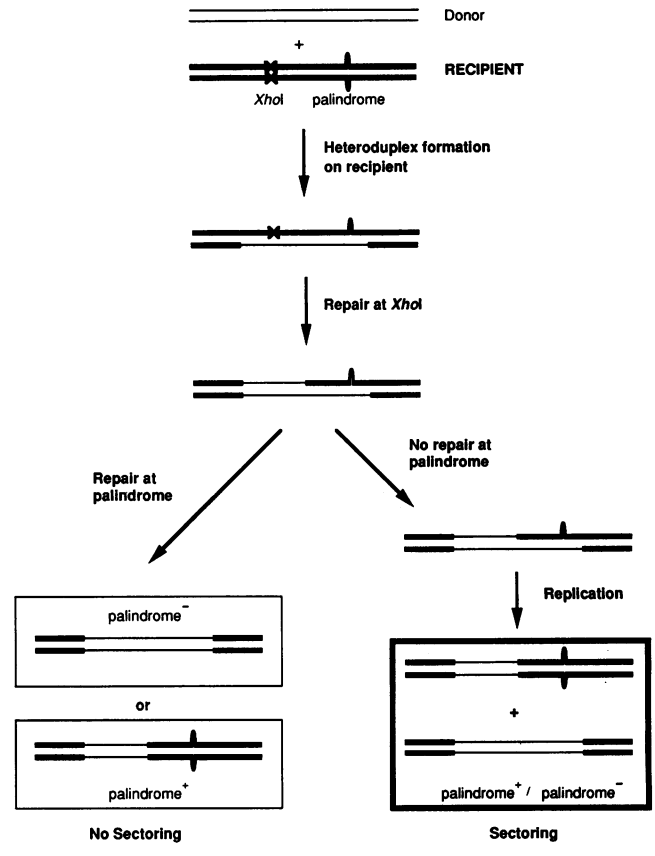

FIG. 3. Rationale for the sectored colony analysis of heteroduplex formation. Heteroduplex formation on the recipient gene is predicted to encompass both the *Xho*I linker mutation and the palindrome polymorphism. Repair at the linker insertion mutation to wild type via the donor strand (gene conversion) must occur to allow recovery of a TK^+ colony. If heteroduplex at the palindrome is repaired either in favor of the donor strand (coconversion) or in favor of the recipient strand (restoration), pure PAL^- or PAL^+ colonies are formed (left pathway). If no repair occurs, heteroduplex resolution by replication in the daughter cells is manifested by a sectored colony (right pathway).

genotypically sectored as a result of replicative resolution of heteroduplex DNA.

We selected for conversion at the XH34 *Xho*I linker mutation site by isolating TK^+ cells and monitored correction at the nearby polymorphic (palindrome) site. We reasoned that if, as depicted in Fig. 3, gene conversion can involve formation of heteroduplex on the recipient strand that includes both the site of the *Xho*I linker mutation and the palindromic site, then with some frequency the mismatch correction machinery will correct the linker insertion mutant site to wild type but leave the silent palindromic site unrepaired. If this heteroduplex remains unrepaired, semiconservative DNA synthesis will generate daughter strands that differ at the polymorphic palindrome site within the last intron of the recipient gene. Assuming that daughter cells remain adjacent and proliferate, a mixed colony (sectored for the palindrome in the recipient gene) is formed. Because the palindrome insertion produces several restriction site differences, the initial signal for a sectored colony is a mixed pattern of cleavage of the recipient gene with each of the diagnostic restriction enzymes (*Xba*I, *Bst*XI, and *Eco*RV; Fig. 2), in contrast to *Xho*I digestion that will be uniform among convertants. To verify colony sectoring, 8 to 10 individual single-cell subclones of each sectored convertant are examined for the presence of the palindrome in their recipient gene. From a true sectored convertant, two classes of subclones are expected: genetically homogeneous subclones that either retain or do not retain the palindrome.

Cell lines for analysis of intrachromosomal recombination. Cell lines to be used for the sectoring assay were generated by direct nuclear microinjection of the recombination substrate (Fig. 1) into Ltk^- cells. Two cell lines, PAL-1 and PAL-4, were chosen for further analysis, since they each contain a single copy of the recombination substrate: a full-length recipient gene with a *Xho*I linker insertion in the coding sequence (XH34) linked to an internal fragment of the chicken *tk* gene. Since the internal fragment lacked essential 5′ and 3′ regulatory sequences, we predicted that single reciprocal exchanges would not produce viable recombinants. Therefore, only gene conversion, or less likely double reciprocal exchange, should generate HAT^r colonies. Unexpectedly, expression at the PAL-1 integration site was sufficient to allow recovery of single-crossover recombinants

(data not shown), but these were excluded from the present analysis.

Recombinant isolation. We obtained recombinants by selecting TK^+ colonies arising from parent lines PAL-1 and PAL-4. Since we were interested only in newly arising colonies, we eliminated preexisting TK^+ cells by growing the parent lines in TFT, a cytotoxic analog of thymidine that requires thymidine kinase activity for its cytotoxic effect. After 10 to 14 days, we plated the parent lines into culture dishes containing a 10-fold excess of thymidine to dilute residual TFT. Dishes were incubated undisturbed for 2 days to allow newly arising TK^+ recombinants to attach and proliferate as single colonies. Subsequently, the medium was replaced with HAT_{10}, a selective medium that eliminates TK^- colonies while maintaining excess thymidine levels.

After TK^+ colonies became clearly visible (~12 days after HAT_{10} selection), we scanned culture dishes for those that contained only a single colony. Single colonies were then isolated and expanded. We froze a portion of the expanded colony and prepared genomic DNA from the remainder.

Sectoring assay. To assay sectoring, we were interested in the molecular configuration of the recipient gene sequence within each colony. To generate HAT^r colonies in our system, the donor gene must correct the *Xho*I insertion in the recipient gene by gene conversion to render the coding region wild type. To determine the genotype of the recipient gene, we amplified the coding region by PCR (29), using primers external to the donor gene fragment. The amplification generated a gene fragment of 1 kb, which we then digested with diagnostic restriction enzymes (*Xba*I, *Eco*RV, and *Bst*XI). Resistance to digestion with *Xho*I was indicative of a successful gene conversion. Digestion with *Xba*I indicated that information at the silent polymorphism was transferred from the donor into the intron of the recipient gene by coconversion (nonparental at the polymorphic site), while resistance to digestion with *Xba*I indicated parental sequence in the intron, i.e., no coconversion. Digestion with restriction enzymes *Bst*XI and *Eco*RV indicated the presence of the original palindrome insertion within the last intron of the recipient gene. A representative analysis of this type is depicted in Fig. 4.

The majority of recombinants fell into either the parental or nonparental class with respect to the polymorphic site (Table 1). Our experiments are designed to seek evidence for heteroduplex DNA at this site; unrepaired heteroduplex DNA should generate sectored recombinant colonies that are part parental and part recombinant in genotype. Such sectoring is manifested by a mixed pattern of cleavage of the recipient fragment with the diagnostic restriction enzymes. We observed five such recombinants in our PCR screen (depicted for one in Fig. 4). To substantiate that the mixed-pattern digestions were not an artifact of the PCR analysis, we verified the sectoring result by Southern blot hybridization analysis of the recombinant colonies (Fig. 5). Since the recombinants appeared to represent bona fide mixed colonies and not a digestion artifact, we tested the potential sectored colonies further by using subclonal analysis.

Subcloning sectored colonies. While the DNA analyses of the original sectored convertants are consistent with a sectored genotype at the palindromic insertion, we used subclone analysis to verify the mixed nature of the colony. If the genotypic sectoring reflects the presence of two subpopulations of cells within the recombinant colony, each deriving from one daughter cell produced by the original recombination event, then representatives of these two subpopulations should be obtained by subcloning.

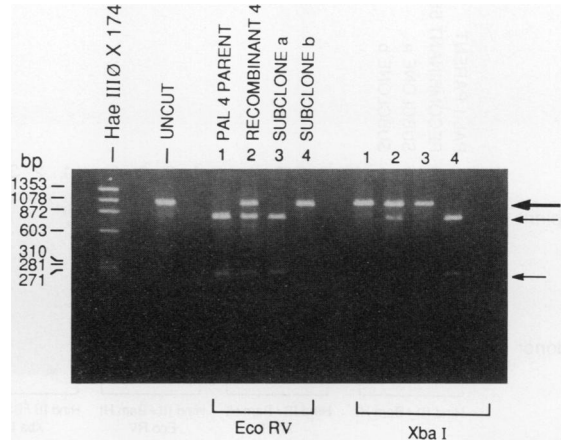

FIG. 4. PCR analysis of sectored recombinant 4. The uncut PCR product is shown (indicated by the larger arrow), and cleavage with restriction enzymes *Eco*RV and *Xba*I should generate the products indicated with smaller arrows. As expected, the PCR product from the parental line (PAL-4; lane 1 in each case) cuts with *Eco*RV and is resistant to cleavage with *Xba*I. The amplified recipient gene sequence in recombinant 4 (lane 2 in each case) shows mixed cleavage with both *Eco*RV and *Xba*I, while purified subclones (lanes 3 and 4 in each case) cleave with only *Eco*RV (a, parental) or *Xba*I (b, nonparental).

Therefore, the original recombinant colonies were subcloned to single-cell isolates. Eight to ten single-cell isolates were picked and expanded for genotype analysis. In accord with prediction, each sectored recombinant produces subclones of both genotypes, parental and nonparental (Table 2). Furthermore, each subclone tests pure in genotype: restriction digestions of the recipient gene were complete with each enzyme, indicating that the original colonies were indeed sectored.

DISCUSSION

We have used a sectored colony assay to detect the formation of hDNA during intrachromosomal gene conversion in mouse cells. The assay involves an intrachromosomal substrate, based on the chicken *tk* gene, which is intended to restrict recovery of TK^+ recombinants to those consistent with gene conversion. We selected for gene conversion at a *Xho*I linker insertion mutation in the recipient gene and monitored a silent restriction site polymorphism deriving from a palindrome insertion within an intron. By digesting the recipient gene with restriction enzymes diagnostic for the two parental configurations at the polymorphism, we could observe the genotype at the unselected site. Sectoring at this site was identified by a mixed pattern of cleavage of the recipient gene with diagnostic restriction enzymes.

Among a total of 125 independent gene convertants from two different single-copy parent lines, we observed five

TABLE 1. Analysis of recombinants

Line	Parental	Nonparental	Sectored	Total
PAL-1	22	30	2	54
PAL-4	21	47	3	71
Total	43	77	5	125

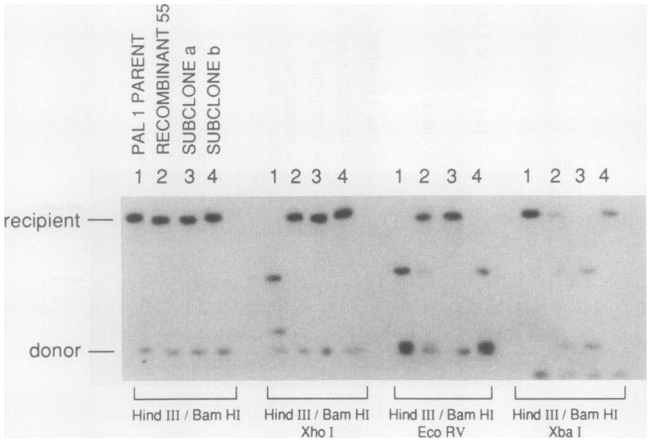

FIG. 5. Southern blot hybridization analysis of sectored recombinant 55. Lanes 1 to 4 in each series of digests represent the parent line (PAL-1), the sectored recombinant 55, subclone a of recombinant 55, and subclone b of recombinant 55, respectively. Donor and recipient gene fragments are flanked by *Hin*dIII and *Bam*HI sites, respectively (first series). The recipient gene in parent line PAL-1 cleaves with *Xho*I, while the recombinants do not (second series). In the third and fourth series, recipient DNA from recombinant 55 shows mixed cleavage with both *Eco*RV (site in recipient) and with *Xba*I (site in donor), while subclones cleave with only *Eco*RV (b, parental) or *Xba*I (a, nonparental). In the fourth series, *Xba*I digestion of recipient sequence from recombinant 55 shows a mixed pattern, while digestion of the donor is complete.

colonies that were sectored for the presence of the 34-bp palindrome (Table 1) but pure in terms of having a wild-type *tk* gene, i.e., lacking the 8-bp *Xho*I insertion. To exclude the possibility that sectoring was an artifact of the restriction endonuclease assay, single-cell subclones of each sectored colony were analyzed with respect to the restriction sites at the palindrome insertion (Table 2). As predicted, each sectored colony produced two classes of subclones; those that were uniformly of the parental genotype (palindrome sites *Eco*RV and *Bst*XI present) and those that were uniformly of the nonparental genotype (*Xba*I site present). Hence, by the criteria defined above, the five recombinants represented sectored colonies.

The simplest explanation for our findings is that each sectored colony represents a single recombination event in which hDNA formed but was not repaired at the site of the palindrome prior to DNA replication. By analogy with fungal recombination, we conclude that the demonstration of sectored colonies indicates that heteroduplex DNA is associated with the process of gene conversion.

At least two alternative explanations for these sectored colonies are possible. First, the sectored colonies might represent the fortuitous and immediately adjacent seeding of two different convertant cells. One convertant would represent coconversion at the palindrome site on the recipient, and one convertant would not. On the basis of the diameter of an average colony and the diameter of the culture wells, we calculate that the probability that the observed sectored colonies actually represent two independent conversion events to be between 1 in 500 and 1 in 2,000. Furthermore, whereas cell line PAL-1 produces both reciprocal and gene conversion recombinants, we do not see mixed reciprocal/conversion products (data not shown). Therefore, we do not favor this "adjacent seeding" explanation.

TABLE 2. Analysis of sectored subclones

Sectored recombinant	Parental	Nonparental
PAL-1		
Recombinant 15	4	5
Recombinant 55	3	5
PAL-4		
Recombinant 4	7	1
Recombinant 58	3	5
Recombinant 70	5	5

A second alternative interpretation invokes two associated recombination events in the same cell on each sister chromatid. If a second independent recombination event occurred in the same cell but on the other sister chromatid with a 10% frequency, then approximately one-half (or 5% of the total recombinants) of these double events would produce a colony sectored for the palindrome (we observed approximately 50% coconversion at the palindrome overall in our study [43:77]). In other words, this second alternative explanation requires a subpopulation of cells that is very "hot" for recombination. An apparent precedence for such a hot subpopulation has been suggested in mitotic yeast cells (12). Indeed, if the sectored colonies observed in the present study actually represent such hot cells, then evidence for hot cells should have been observed at a 10% frequency in our previous investigations of intrachromosomal recombination. For example, for substrates with two different *Xho*I linker insertion alleles of the herpes simplex virus *tk* gene oriented as direct or inverted repeats, hot cells would be manifested by TK^+ cells that have two wild-type genes representing conversion of both mutant alleles. In over 400 recombinants examined in our laboratory, no such double wild-type recombinants were detected. For this reason, we do not favor the "hot cell" explanation. One possible means to address whether the observed sectored colonies truly represent unrepaired hDNA is to perform similar experiments in a mismatch repair-deficient mammalian cell line, in which the frequency of sectoring should increase.

Obviously, the sectoring assay described here is informative only when hDNA at the diagnostic site is not repaired prior to DNA replication. Therefore, we chose as a marker a perfect palindrome based on the studies of Nag et al. (25) demonstrating that perfect palindromes inhibit hDNA repair during meiotic recombination in yeast cells. In their studies, hDNA at the palindromic site remained unrepaired with 80% frequency. Our frequency of sectoring which represents unrepaired hDNA is much lower, 5%. We offer two possible explanations for this difference. One is that in our system, selection for TK^+ cells requires conversion (correction) at the linker insertion mutation that is located only 93 bp from the diagnostic palindrome insertion. Certain studies in fungi show that repair is epistatic (or dominant) to no repair (14). Therefore, assuming that hDNA spans both the *Xho*I mutant site and the site of the palindrome polymorphism, then correction at the *Xho*I mutant site might force correction at the palindromic site and hence reduce the frequency of sectoring. If a nonselectable assay were used, we would expect a higher level of sectoring at the palindromic insertion. A second reason for the low apparent frequency is that mismatch repair may be an efficient process in mammalian cells, as studies of extrachromosomal heteroduplex repair suggest. We have recently made the suggestion that intrachromosomal reciprocal recombination most frequently occurs after DNA replication (5). If this is also true for gene

conversion, then heteroduplex arising in G_2 must persist for an entire cell cycle until the subsequent round of DNA replication in order to obtain sectoring.

The results with yeast cells showed clearly that a palindromic insertion is much less likely to be corrected than a nonpalindromic insertion of the same size (25). To address this point regarding palindromic versus nonpalindromic insertions in mammalian cells and to inquire into correction of other types of mismatches (e.g., G/T versus G/G) will require modification of our present system (e.g., a cytochemical staining assay) so that many more independent recombinant colonies could be easily examined for sectoring. Our strategy could provide a logical extension of experiments in which cells or cell extracts process preformed heteroduplexes with different efficiencies (6).

Our results, while supporting the existence of heteroduplex DNA, do not address the mechanism of heteroduplex formation. One mechanism, envisioned originally by Meselson and Radding (22), involves the invasion of a single-strand joint on the donor strand. Other mechanisms imagine an initiation event deriving from a double-strand break in the recipient duplex molecule (30). Such an initiation event can suffer two potential fates: enlargement to a gap with staggered ends or recision of strands of opposite polarity and subsequent annealing. The latter process results in loss of genetic material, a nonconservative form of recombination. The results from extrachromosomal recombination studies favor such a model (20), but previous studies suggest that intrachromosomal recombination is frequently conservative (4) and render a recision/annealing model unlikely.

In summary, we believe that our results showing segregation to daughter cells of an unselected genetic marker (a 34-bp perfect palindrome) demonstrate the formation of heteroduplex DNA during intrachromosomal gene conversion in mouse cells. However, these experiments do not address the question of whether the gene conversion (repair) that occurred at the selected site, the *Xho*I linker insertion mutation, actually involved repair of heteroduplex DNA. For example, another process such as double-strand gap repair might be responsible for the removal of the *Xho*I linker insertion mutation. On the basis of our present results and those of previous studies that indicated a significant difference in gene conversion efficiencies for a single base insertion versus a single base deletion (19), we favor a direct role for heteroduplex DNA formation and processing in intrachromosomal gene conversion in mammalian cells.

ACKNOWLEDGMENTS

We thank Rodney J. Rothstein for encouraging the development of a sectoring assay, Thomas D. Petes for pointing out the merits of a palindrome insertion, and Charles M. Radding for helpful comments on the manuscript.

This work was supported by NIH grants GM32741-08 and GM45413-01 to R.M.L.

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