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Extrachromosomal and Chromosomal Gene Conversion in Mammalian Cells

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We constructed substrates to study gene conversion in mammalian cells specifically without the complication of reciprocal recombination events. These substrates contain both an insertion mutation of the neomycin resistance gene (*neoX*) and an internal, homologous fragment of the *neo* gene (*neo-526*), such that gene conversion from *neo-526* to *neoX* restores a functional *neo* gene. Although two reciprocal recombination events can also produce an intact *neo* gene, these double recombination events occur much less frequently than gene conversion events in mammalian cells. We used our substrates to characterize extrachromosomal gene conversion in recombination-deficient bacteria and in monkey COS cells. Chromosomal recombination was also studied after stable integration of these substrates into the genome of mouse 3T6 cells. All extrachromosomal and chromosomal recombination events analyzed in mammalian cells resulted from gene conversion. Chromosomal gene conversion events occurred at frequencies of about 10^{-6} per cell generation and restored a functional *neo* gene without overall effects on sequence organization.

The recombinational machinery in mitotically dividing mammalian cells is believed to play a fundamental role in determining genome organization and in the maintenance of DNA sequence homogeneity among members of repeated gene families (1, 5–7, 14, 15). The homology-dependent and -independent processes catalyzed by this recombinational machinery have not been analyzed systematically until recently, when expression vectors carrying mutant or truncated copies of selectable marker genes provided the basic tools with which such events could be analyzed.

Recent experiments on chromosomal homologous recombination in a number of laboratories have relied on the use of either overlapping truncated fragments or mutant copies of the herpesvirus thymidine kinase (*tk*) (9–11) or neomycin resistance (*neo*) gene (16, 20) to detect recombination events which restore one or more copies of the selected marker gene. Although these studies have measured recombination frequencies and characterized various reciprocal and nonreciprocal intrachromosomal recombination events, attempts to estimate the true frequencies of these events have been complicated by the ability of the recombination substrates to undergo both reciprocal and nonreciprocal recombination. Thus, it has been difficult to assign frequencies to each type of event independently without doing fluctuation tests to estimate frequencies and characterizing sufficiently large numbers of independently derived recombination products from different parental cell lines to determine the relative proportions of reciprocal and nonreciprocal recombination. With these caveats in mind, the impression one gets from the published literature is that in some cell lines (11, 16) gene conversion occurs 80% of the time, while reciprocal recombination occurs 20% of the time, whereas in others, reciprocal recombination predominates over nonreciprocal recombination (16).

We constructed substrates to study chromosomal gene conversion in mammalian cells specifically without the complication of reciprocal recombination events. The substrates contained a mutated, nonfunctional *neo* gene (*neoX*) and a homologous 526-base-pair (bp) internal fragment of the *neo* gene (*neo-526*) separated by a functional transcription unit expressing the bacterial xanthine-guanine phosphoribosyltransferase gene (*gpt*). Gene conversion, or nonreciprocal transfer of genetic information from *neo-526* to *neoX*, restores a functional *neo* gene whose gene product confers resistance to neomycin in bacteria or G418 in mammalian cells. Two reciprocal recombination events can also produce an intact *neo* gene, but since the frequency of single-crossover events in the chromosome is 10^{-6} to 10^{-8} per cell generation (20), double-reciprocal recombination events are extremely rare relative to the frequency of nonreciprocal recombination.

Using these and other related substrates, we characterized recombination frequencies and products of recombination between direct and inverted repeat configurations of the *neoX* and *neo-526* segments. We studied these in recombination-deficient bacteria, in monkey COS cells, where they replicate extrachromosomally, and after stable integration into the genome of mouse 3T6 cells. In bacteria, the substrates with the *neoX* and *neo-526* sequences arranged as direct repeats regenerated the *neo* gene both by double-reciprocal and nonreciprocal recombination, whereas substrates with these segments arranged as inverted repeats showed only gene conversion. In contrast, all the extrachromosomal and chromosomal recombination events analyzed in COS or 3T6 cells resulted from gene conversion. Chromosomal gene conversion, as measured by fluctuation tests, occurred at frequencies of about 10^{-6} per cell generation. Analysis of the gene conversion events indicated that, unlike the reciprocal recombination events we and others have characterized previously (9–11, 16, 20), they occurred without overall effects on sequence organization.

MATERIALS AND METHODS

Structures of recombination substrates. The recombination substrates used in this study contain a mutated, nonfunctional *neo* gene, designated *neoX*, and a 526-bp, nonfunctional internal fragment of the *neo* gene, *neo-526* (see Fig. 1). The substrates, pGCD1 and pGCI1, were constructed from the plasmid ptkgptSVneo, which has a complete transcription unit (flanked by *Bam*HI restriction endonuclease cleavage

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sites) comprising the neomycin resistance gene from Tn5 linked to transcription signals (promoter, small-t intron, and polyadenylation signal) from the simian virus 40 early region (19). The molecule ptkgptSVneo also contains the ampicillin resistance gene and origin of DNA replication from pBR322 and the bacterial *gpt* gene linked to the *tk* promoter and the simian virus 40 small-t intron and polyadenylation signal.

To construct substrates for gene conversion, we mutated the *neo* gene in ptkgptSVneo by inserting a 10-bp synthetic *Xho*I linker in the *neo* coding region at the *Bal*I site (nucleotide 1759 in reference 2), thus creating *neoX* (see Fig. 1). An internal fragment of the *neo* gene was obtained by digesting pSV2neo (19) with restriction enzymes *Xma*III and *Nco*I and isolating the 526-bp fragment that overlaps the *Xho*I site in *neoX*. The ends of this DNA fragment, referred to as *neo-526*, were made blunt with *Escherichia coli* DNA polymerase I, ligated to synthetic *Eco*RI linkers, and digested with *Eco*RI. *neo-526* was then inserted in either orientation into the *Eco*RI site downstream of *neoX*, creating pGCD1 and pGCI1, which contain *neo-526* in a direct or inverted configuration with respect to *neoX* (see Fig. 1A). Plasmids pGCD2 and pGCI2 (see Fig. 1B) are similar to pGCD1 and pGCI1 but lack the *gpt* transcription unit.

Recombination frequencies. The frequencies of recombination events between *neo-526* and *neoX* to produce a functional *neo* gene were determined in *E. coli* HB101, monkey COS cells, and mouse 3T6 cells as previously described (13, 20). Briefly, Luria-Delbruck fluctuation tests (12) were used to determine recombination frequencies for pGCD1, pGCI1, pGCD2, and pGCI2 in HB101 and for pGCD1 and pGCI1 in 3T6 cells. Fluctuation tests were also used to measure the rate at which *neoX* spontaneously reverted to a functional *neo* gene in the absence of *neo-526*. Recombination frequencies in COS cells were determined by transfecting these cells with the recombination substrates, extracting low-molecular-weight DNA after 1 to 2 days by the method of Hirt (4), and transforming HB101 with the extracted DNA. The ratios of Neo^r to Amp^r colonies were used to calculate recombination frequencies in this case. All the recombination frequencies reported are the averages of 2 to 4 independent experiments.

DNA transfections, genomic DNAs, Southern blots, and DNA probes. Mouse 3T6 cells and monkey COS cells were transfected as previously described (13, 20). Extraction of DNA, electrophoresis, and transfer to nitrocellulose paper were performed by standard procedures (18, 21). All filters were hybridized with a DNA probe that was specific for the *neo* gene. Labeled probe was made by nick translation of the 1,339-bp *Hin*dIII-*Nru*I DNA fragment from pSV2neo (19).

RESULTS

Neo^r recombination products. In the recombination substrates described above (Fig. 1), intra- or intermolecular gene conversion (nonreciprocal recombination) between *neo-526* and *neoX* generates a functional *neo* gene lacking the *Xho*I site and capable of conferring resistance to neomycin in bacteria or to G418 in mammalian cells. For all substrates, gene conversion yields a plasmid (or integrated plasmid sequences in 3T6 cells) containing both the intact *neo* gene and *neo-526*. Reciprocal recombination between *neo-526* and *neoX* can also generate a functional *neo* gene, although this requires two recombination events between *neo-526* and *neoX*, one upstream (174 bp of homology) and the other downstream of the *Xho*I linker (352 bp of homology). Intramolecular double-reciprocal recombination yields a plasmid containing an intact *neo* gene as well as an *Xho*I site in *neo-526* (shown schematically in Fig. 2).

Recombination in bacteria. To measure recombination frequencies in bacteria, we transformed *E. coli* HB101 (*recA*) to ampicillin resistance with each of the recombination substrates (Fig. 1) as well as with pSV2neoX, which is similar to pGCD2 and pGCI2 but lacks the *neo-526* segment. We included pSV2neoX to determine the background rate at which *neoX* generated a functional *neo* gene in the absence of recombination. For each plasmid, Amp^r Neo^s colonies were isolated and Luria-Delbruck fluctuation tests (12) were performed (Table 1). Recombination frequencies were similar for all four recombination substrates (1.4×10^{-6} to 2.9×10^{-6} per cell generation) and 2 orders of magnitude higher than the background reversion frequency for pSV2neoX (2.2×10^{-8} per cell generation).

Analysis of bacterial recombination products. Recombination products were analyzed by isolating plasmid DNA from independent Neo^r colonies that arose from pGCD2 and pGCI2. The mode of recombination (i.e., gene conversion or double-reciprocal recombination) was easily determined by restriction enzyme analysis of these plasmid DNAs (Table 1). Digestion of pGCD2 and pGCI2 with restriction enzymes *Xho*I and *Bam*HI yielded DNA fragments of 1,772 and 4,482 bp. For both plasmids, digestion of gene conversion products with *Xho*I and *Bam*HI yielded only a 6,254-bp fragment, since these products lacked an *Xho*I site (Fig. 2). In contrast, the product of intramolecular double-reciprocal recombination had an *Xho*I site in the *neo-526* segment, so that digestion of this product from pGCD2 with *Xho*I and *Bam*HI yielded 925- and 5,329-bp DNA fragments (Fig. 2). pGCD2 underwent primarily (75%) intramolecular double-reciprocal recombination, with gene conversion occurring in 25% of the cases analyzed (Table 1). pGCI2, however, underwent only gene conversion, consistent with our earlier studies showing that intramolecular reciprocal recombination did not occur between inverted repeats in *E. coli* (13). It is impossible to determine whether the gene conversion products seen for pGCD2 and pGCI2 arose from inter- or intramolecular events.

Neo^r products were also analyzed for the control plasmid pSV2neoX (Table 1). Of 12 independent products analyzed, only 1 had lost the *Xho*I site in *neoX*. All other Neo^r plasmids retained the *Xho*I site and presumably had undergone a second site mutation to generate a functional *neo* gene (Fig. 2). The Neo^r phenotype was localized to the plasmid and was not due to suppressor mutations in HB101, because the plasmid DNA maintained its Neo^r property when transfected into another batch of competent HB101 cells. Since the 10-bp *Xho*I linker insertion mutation acts by shifting the reading frame of the *neo* gene, it is likely that many of the second site mutations were simple frameshift suppressor mutations near the *Xho*I site. The precise location of these mutations was not pursued further.

Gene conversion in COS cells. Extrachromosomal recombination was analyzed in monkey COS cells (3), which support the replication of plasmids containing the simian virus 40 origin of replication. Recombination frequencies were determined by transfecting COS cells with pGCD2, pGCI2, or the control plasmid pSV2neoX, extracting low-molecular-weight DNA after 1 to 2 days (4), and using the extracted DNA to transform HB101. Ratios of Neo^r to Amp^r colonies were used to calculate recombination frequencies (Table 2). Background rates in HB101 were determined by direct transformation of the substrates into HB101 without prior passage through COS cells.

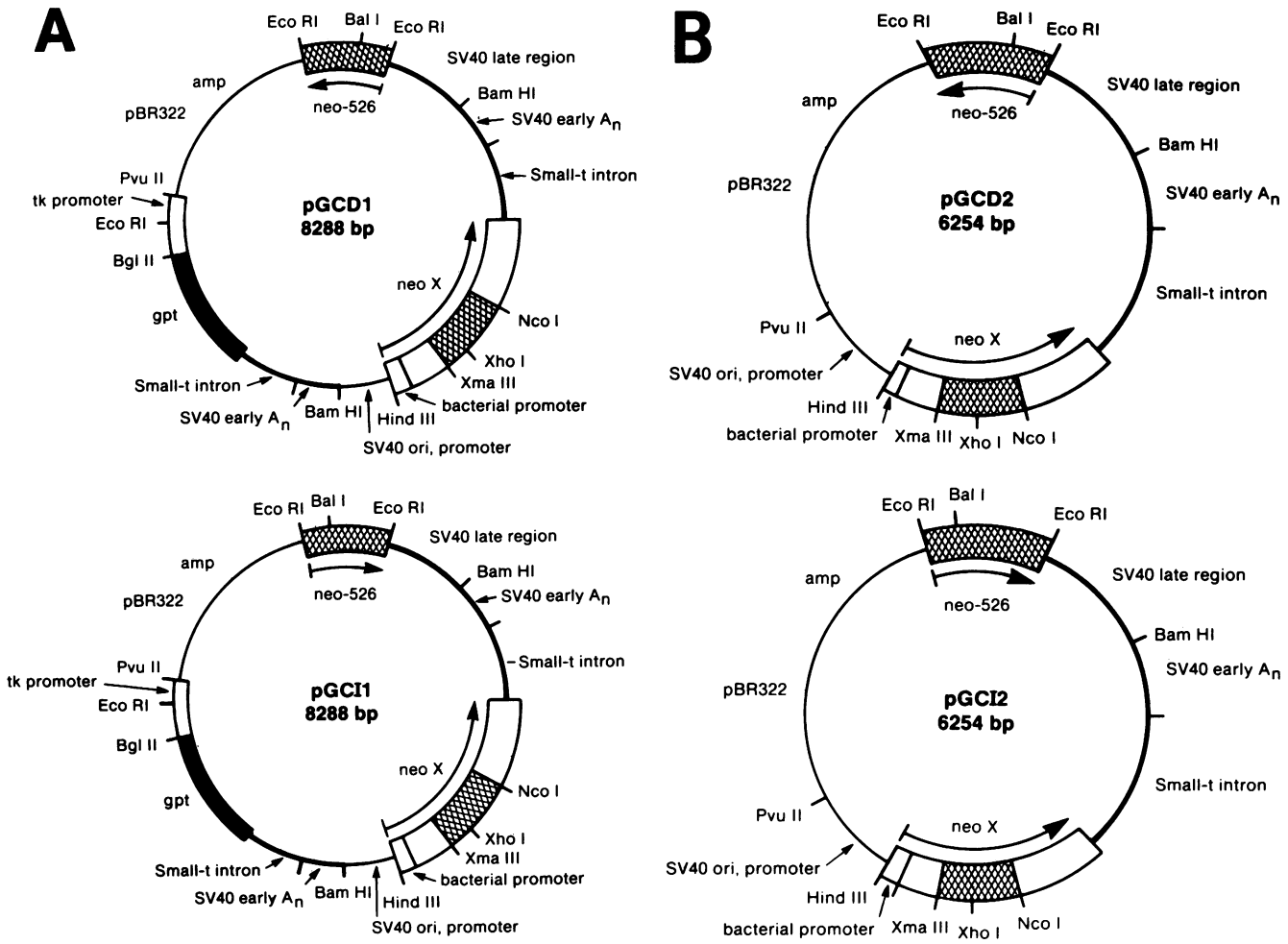

FIG. 1. Gene conversion substrates. (A) pGCD1 and pGCI1 each contain a mutated *neo* gene (*neoX*) and an internal fragment of the *neo* coding region (*neo-526*), which share 526 bp of homology (hatched) and are present in a direct (pGCD1) or inverted (pGCI1) configuration. The plasmids also contain the ampicillin resistance gene and origin of DNA replication from pBR322, a complete *gpt* transcription unit, and the simian virus 40 early promoter and viral origin of DNA replication. (B) pGCD2 and pGCI2 are similar to pGCD1 and pGCI1 but lack the *gpt* transcription unit.

Recombination frequencies in COS cells were generally quite low, in the range of 2.6×10^{-4} to 5.6×10^{-4}. Greater than 95% of the products analyzed arose from gene conversion, indicating that the frequencies shown in Table 2 were in fact gene conversion frequencies. These frequencies most likely reflect events that took place in COS cells, since the frequencies for both pGCD2 and pGCI2 were at least 10-fold higher than the background rates in HB101. Moreover, the recombination products obtained after passage of the recombination substrates through COS cells were quite distinct from those obtained by recombination in bacteria (Tables 1 and 2). This low frequency of extrachromosomal gene conversion is in contrast to the high frequency of single-crossover events we have previously detected in COS cells (13).

To ensure that our results were not affected by the

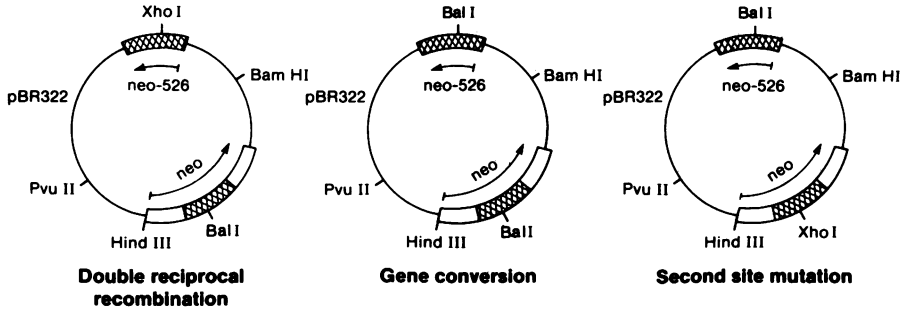

FIG. 2. Neo^r recombination products for pGCD2.

TABLE 1. Recombination in *E. coli*

Plasmid	Background reversion frequency/cell generation	Recombination frequency/cell generation	Neo^r recombination products[a]
pGCD1		2.7×10^{-6}	ND[b]
pGCI1		1.4×10^{-6}	ND
pGCD2		2.9×10^{-6}	23/32—double-reciprocal recombination 8/32—gene conversion 1/32—second site mutation
pGCI2		2.0×10^{-6}	21/21—gene conversion
pSV2neoX	2.2×10^{-8}		11/12—second site mutation 1/12—mutation at *Xho*I site

[a] Neo^r recombination products for pGCD2 are shown in Fig. 2.
[b] ND, Not determined.

presence of plasmid DNA that had not been taken up by COS cells, we treated the extracted DNAs with the restriction enzyme *Dpn*I. Because *Dpn*I cleaves only methylated DNA, it does not cleave DNA that has replicated in COS cells, but it does cleave all input DNA. Gene conversion frequencies as determined by bacterial transformations were unchanged after *Dpn*I treatment of Hirt DNAs.

Construction of 3T6 cell lines containing the substrates. To study chromosomal gene conversion, we transfected 3T6 cells with pGCD1, pGCI1, or the control plasmid ptkgptSVneoX, which lacks the *neo-526* segment. After selection for *gpt* expression, seven *gpt*-positive lines were chosen for further study: four from pGCD1, one from pGCI1, and two from ptkgptSVneoX (Table 3). All lines remained *gpt* positive even after growth in nonselective medium for over 30 generations, and Hirt supernatants from these cell lines showed no evidence of extrachromosomal DNA. Furthermore, the pattern of hybridizing chromosomal DNA segments was unchanged on Southern blot analysis (see below), indicating that the seven cell lines studied had stably integrated copies of the transfected DNAs.

Gene conversion frequencies in 3T6 cells. We performed fluctuation tests (12) on all 3T6 cell lines to estimate the frequency of chromosomal recombination events leading to production of a functional *neo* gene. Recombination frequencies were similar for GCD1 and GCI1 cell lines, ranging from 6.6×10^{-7} to 2.7×10^{-6} per cell generation (Table 3). We presumed that the events detected by this method were primarily gene conversion, because two reciprocal recombination events between *neo-526* and *neoX* are required to generate a functional *neo* gene, and our earlier studies had indicated that the frequency of chromosomal single crossovers in 3T6 cells was 10^{-8} to 10^{-6} per cell generation (20). We also did fluctuation tests on 3T6/SVneoX cell lines 8-3b and 8-5d (Table 3) to determine the background reversion rate of *neoX* in 3T6 cells in the absence of *neo-526*. Surprisingly, both 3T6/SVneoX clones spontaneously gave rise to G418^r colonies, but only at frequencies of about 4×10^{-8}, 16- to 67-fold lower than for GCD1 and GCI1 clones.

Structure of integrated DNA. Genomic DNAs from the pGCD1 and pGCI1 clones were digested with *Bam*HI, *Eco*RI, and *Xho*I (alone or in combinations of two enzymes) and analyzed by Southern blots hybridized with the *neo* probe. Digestion of these genomic DNAs with *Bam*HI followed by hybridization with the *neo* probe should yield the *neoX* transcription unit as a 2,682-bp fragment, whereas *Eco*RI digestion should excise the *neo-526* segment as a 526-bp fragment. All GCD1 and GCI1 clones showed these diagnostic fragments, as demonstrated for 3T6/GCI1/7-4h in Fig. 3. Digestion of 7-4h with *Bam*HI showed the 2,682-bp *neoX* fragment (Fig. 3, lane 1), whereas the *Bam*HI-*Eco*RI digest showed both the 2,682-bp and *neo-526* fragments (Fig. 3, lane 7). Besides these diagnostic bands (see also Fig. 4), the *Bam*HI digest of 7-4h had two other hybridizing fragments—one at 5.6 kilobases (kb) (containing plasmid and *neo-526* sequences) and the other at 4.7 kb (consisting of *neoX* and flanking chromosomal sequences) (Fig. 3, lane 1). The *Bam*HI-*Eco*RI digest of 7-4h also showed a junction fragment at about 4.6 kb (Fig. 3, lane 7).

Since *neoX* has an *Xho*I site (Fig. 4), digestion of 7-4h DNA with *Bam*HI and *Xho*I resulted in digestion of the 2,682-bp *Bam*HI fragment to 910- and 1,772-bp segments and in cleavage of the 4.7-kb *Bam*HI fragment to 910- and 3.8-kb fragments (Fig. 3, lane 2).

The 3T6/GCD1 and 3T6/SVneoX cell lines (Table 3) were analyzed in a similar fashion and found to contain 2 to 5 copies of integrated plasmid sequences (data not shown). Digestion of genomic DNAs from these cell lines with *Sac*I, an enzyme that does not cut within the input plasmid DNAs,

TABLE 2. Gene conversion in COS cells[a]

Plasmid	Background rate in HB101	Days post-infection	Gene conversion frequency	Neo^r recombination products[b]
pGCD2	2×10^{-5}	1	2.6×10^{-4}	7/8—gene conversion
		2	2.8×10^{-4}	
pGCI2	2×10^{-5}	1	3.1×10^{-4}	16/16—gene conversion
		2	5.6×10^{-4}	
pSV2neoX	$<1 \times 10^{-5}$	1	$<4 \times 10^{-5}$	ND[c]
		2	$<4 \times 10^{-5}$	

[a] Gene conversion frequencies were measured by ratios of Neo^r to Amp^r colonies obtained after transformation of HB101 with Hirt DNAs from COS cells infected with various plasmids.
[b] Schematic diagrams of possible Neo^r recombination products are shown in Fig. 2. All the recombination products analyzed were obtained independently from a number of separate transfections of COS cells with pGCD2 or pGCI2.
[c] ND, No Neo^r colonies were detected.

TABLE 3. Chromosomal gene conversion frequencies in 3T6 cells[a]

Cell line	Background reversion frequency/cell generation	Avg gene conversion frequency/cell generation
3T6/GCD1/4-2b		2.2×10^{-6}
3T6/GCD1/4-3c		2.7×10^{-6}
3T6/GCD1/5-3c		6.6×10^{-7}
3T6/GCD1/5-2a		1.8×10^{-6}
3T6/GCI1/7-4h		6.7×10^{-7}
3T6/SVneoX/8-3b[b]	4.1×10^{-8}	
3T6/SVneoX/8-5d[b]	4.0×10^{-8}	

[a] Gene conversion frequencies were determined by fluctuation tests as described in the text.
[b] These lines contained integrated copies of only the *neoX* and not the *neo-526* segment.

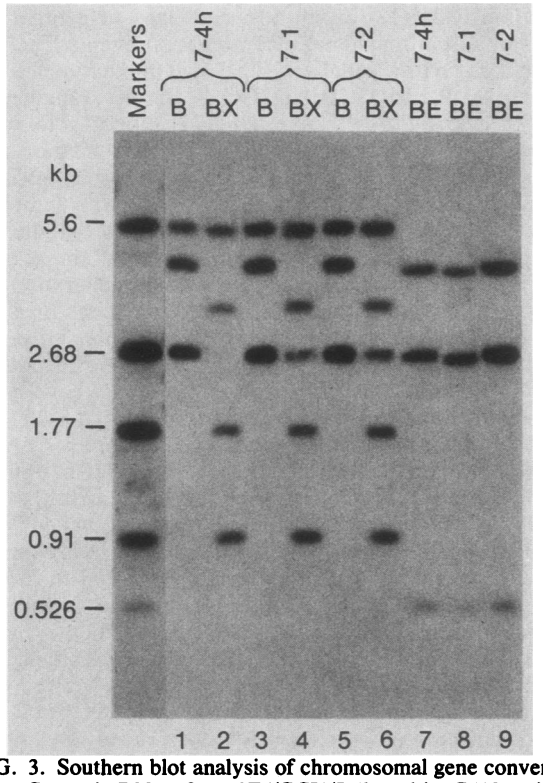

FIG. 3. Southern blot analysis of chromosomal gene conversion events. Genomic DNAs from 3T6/GCI1/7-4h and its G418-resistant derivatives 7-1 and 7-2 were digested with *Bam*HI (B), *Bam*HI and *Xho*I (BX), or *Bam*HI and *Eco*RI (BE) and hybridized with a *neo* probe. The *neoX* transcription unit is present as a 2.68-kb *Bam*HI fragment, and *neo-526* is a 526-bp *Eco*RI fragment.

yielded one hybridizing fragment, indicating one site of integration in all cases except one. Only 3T6/SVneoX/8-5d appeared to have plasmid sequences integrated at two chromosomal locations. The organization of integrated sequences in the four 3T6/GCD1 lines differed from that in 3T6/GCI1/7-4h (Fig. 3 and 4), primarily because integration into cellular sequences had occurred through different points on the input DNAs. Thus, these lines showed different patterns of plasmid-chromosome junction fragments. Nevertheless, Southern blots of DNAs from all four GCD1 lines revealed the *neoX* transcription unit as a 2,682-bp *Bam*HI fragment that was completely digested by *Xho*I to 910- and 1,772-bp segments.

Analysis of chromosomal recombination products. Several independent G418^r colonies that arose from 3T6/GCD1, 3T6/GCI1, and 3T6/SVneoX cell lines were grown for further analysis. As expected from our experimental design, G418^r colonies from 3T6/GCD1 and 3T6/GCI1 appeared to be generated solely by gene conversion. For example, 7-1 and 7-2 (Fig. 3) are two independent G418^r colonies that arose from 3T6/GCI1/7-4h. Digestion of genomic DNAs from 7-1 and 7-2 with *Bam*HI or *Bam*HI and *Eco*RI produced hybridization patterns identical to that for 7-4h (compare Fig. 3, lanes 1, 3, and 5 as well as lanes 7, 8, and 9), as predicted for gene conversion. Digestion of 7-1 and 7-2 DNAs with *Bam*HI and *Xho*I, however, revealed the presence of an *Xho*I-resistant 2,682-bp fragment (Fig. 3, lanes 4 and 6) not seen in 7-4h (Fig. 3, lane 2). The 2,682-bp fragment in lanes 4 and 6 of Fig. 3 cannot be derived by incomplete digestion with *Xho*I, because the digestion of the diagnostic 4.7-kb band obtained with *Bam*HI to 3.8- and 0.9-kb bands in these lanes indicates complete digestion with *Xho*I. This 2,682-bp fragment therefore represents an intact *neo* gene that lacks an *Xho*I site and is responsible for the G418^r phenotype of these cell lines. The presence and intensities of the 910- and 1,772-bp *Bam*HI-*Xho*I bands in 7-1 and 7-2 indicate that *neoX* in only one of the three amplified units in 7-4h was gene converted.

If lines 7-1 and 7-2 had acquired a functional *neo* gene by gene conversion rather than double-reciprocal intrachromosomal recombination, there should not have been an *Xho*I linker in any of the *neo-526* segments in these cells. This was shown by the fact that *Bam*HI-*Xho*I double digests of genomic DNAs from lines 7-4h, 7-1, and 7-2 all had 5.6-kb *Bam*HI fragments (containing *neo-526*) with no *Xho*I sites in these fragments (Fig. 3, lanes 2, 4, and 6).

Two additional G418^r clones derived from 3T6/GCD1/4-2b and four from 3T6/GCD1/5-3c were analyzed in a similar manner and shown to have arisen by gene conversion (data not shown). Several G418^r clones derived from 3T6/SVneoX/8-3b and 3T6/SVneoX/8-5d were also analyzed by Southern blots and shown to contain an *Xho*I-resistant 2,682-bp fragment (in a *Bam*HI-*Xho*I digest) representing a functional *neo* gene (data not shown). Since these lines result from reversion of the *neoX* gene (Table 3) in the absence of the *neo-526* segment, they may contain small deletions removing the *Xho*I site or have acquired a second frameshift mutation within the *Xho*I site. It should be noted however, that these reversion events occur at a frequency that is 16- to 67-fold lower than the gene conversion events described earlier.

DISCUSSION

Unequal sister chromatid exchange and gene conversion have been implicated in the maintenance of DNA sequence

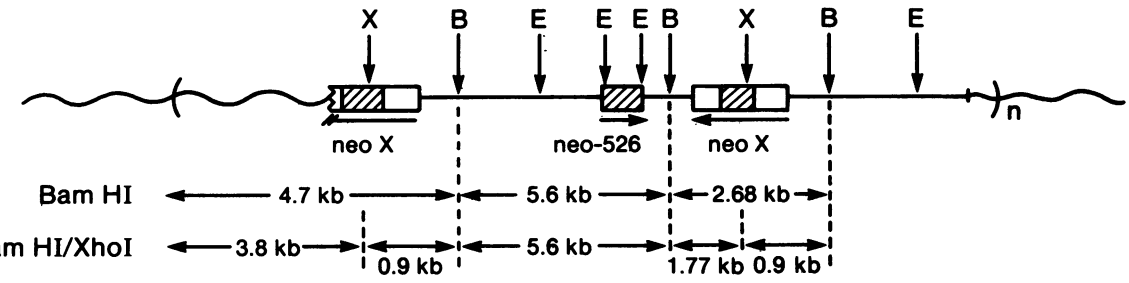

FIG. 4. Arrangement of integrated DNA in 3T6/GCI1/7-4h. The DNA shown in parentheses is amplified three times. Restriction enzyme sites for *Bam*HI (B), *Xho*I (X), and *Eco*I (E) are shown. *Bam*HI and *Bam*HI-*Xho*I restriction fragments hybridizing with the *neo* probe are shown below. Gene conversion results in the loss of at least one *Xho*I site. $n = 3$.

homogeneity among members of multigene families (1, 10). Whereas unequal sister chromatid exchange can make tandemly repeated genes homogeneous, gene conversion can homogenize sequences both within and between chromosomes. Furthermore, unlike unequal sister chromatid exchange, which can alter gene dosage (by deleting genes, for example) gene conversion can occur without gross alterations in genome organization. In this study, we therefore focused on characterizing the frequencies and products of extrachromosomal and chromosomal gene conversion in mammalian cells.

The substrates we used contain both a linker insertion mutation of the *neo* gene (*neoX*) and an internal fragment of the wild-type *neo* gene (*neo-526*). Though these substrates can recombine to generate a functional *neo* gene either by gene conversion or double-reciprocal (intra- or intermolecular) recombination, our analysis is consistent with our presumption that gene conversion would predominate over double-reciprocal recombination. In addition, since our substrates are based on dominant selectable marker genes, thay can be used in a variety of cell types, including those of human origin.

Recombination of substrates in bacteria. Since the recombination substrates were initially propagated in bacteria and because our assay for extrachromosomal recombination in COS cells relied on screening for Neo^r recombinants in recombination-deficient bacteria, we studied the frequencies and products of recombination in HB101. Even though the *neoX* gene had a 10-bp *Xho*I linker inserted into the coding region of the neomycin resistance gene, it reverted spontaneously (primarily by second-site mutations in the plasmid and presumably elsewhere within the *neoX* gene) to generate Neo^r colonies at a frequency of 2.2×10^{-8} per cell generation. Recombination between the *neoX* and *neo-526* segments, however, occurred at 100-fold higher frequencies, whether the homologous regions were arranged as direct or inverted repeats (Table 1). The recombination products generated by pGCD2 (containing direct repeats) were quite distinct from those generated by pGCI2 (containing inverted repeats). Thus, whereas pGCD2 showed about 75% intramolecular double-crossover and 25% gene conversion products, pGCI2 showed only gene conversion. This result is consistent with our previous studies (13), which showed that plasmids with homologous repeats in an inverted configuration do not undergo intramolecular recombination events for reasons we do not yet understand.

Extrachromosomal recombination in COS cells. In COS cells, the recombination frequencies for pGCD2 and pGCI2 were quite low, in the range of 2×10^{-4} to 6×10^{-4}. These frequencies are about 10-fold lower than the frequencies of extrachromosomal single-crossover events we have detected previously for similar plasmids (e.g., DR2) in COS cells (13). The frequencies obtained for pGCD2 and pGCI2 in COS cells are 13- to 28-fold higher than the background recombination frequency of 2×10^{-5} obtained during transformation of HB101 with the substrates (Table 2) and at least 25- to 56-fold higher than the reversion frequency for the *neoX* gene.

The extrachromosomal recombination products obtained from COS cells were also distinct from those observed in HB101 (compare Tables 1 and 2). Both pGCD2 and pGCI2 appeared to undergo only gene conversion to generate a functional *neo* gene. The gene conversion products analyzed were identical to one of the products of intermolecular, double-reciprocal recombination. Since only the Neo^r recombination products were scored in our assay, it is difficult to state definitively that only intramolecular gene conversion occurred in COS cells. When *neoX* and *neo-526* were on different plasmids and cotransfected into COS cells, however, the intermolecular recombination frequency was only 4×10^{-5} (data not shown). Since this value is 6 to 14 times lower than the frequencies for pGCD2 and pGCI2 (Table 2), it seems likely that pGCD2 and pGCI2 underwent intramolecular gene conversion primarily. This result is not surprising, because our earlier studies indicated that molecules similar to pGCD2 and pGCI2 underwent single intramolecular crossovers at frequencies of 10^{-3} and intermolecular reciprocal recombination at frequencies of 10^{-4}. Gene conversion, therefore, occurs more frequently than intra- or intermolecular double-crossover events.

Chromosomal gene conversion in mouse cells. The major goal of this study was to characterize chromosomal gene conversion in mammalian cells. Our substrates are uniquely suited to this purpose, because, based on our previous estimates of frequencies for single-crossover events (10^{-6} to 10^{-8} per cell generation), double crossovers should be extremely rare. Thus, most, if not all, recombination events detected should be due to gene conversion rather than double-reciprocal recombination occurring either within a chromatid or unequally between sister chromatids.

In 3T6 cells, gene conversion occurred at frequencies of 6.6×10^{-7} to 2.7×10^{-6} per cell generation. The average gene conversion frequency (Table 3) was approximately fivefold higher than the average single-crossover frequency we have measured earlier (20). This result is consistent with those of Liskay et al. (10, 11), who found that gene conversion between defective *tk* genes occurred more frequently than reciprocal recombination.

The gene conversion frequencies in Table 3 were 16- to 67-fold higher than the background reversion frequency of the *neoX* gene. The events characterized were all intrachromosomal or between sister chromatids and not interchromosomal, because all the cell lines analyzed had the substrate molecules at a single chromosomal location. Data from Southern blots also support the view that gene conversion was responsible for the generation of a functional *neo* gene, because no rearrangements of the integrated DNA substrates were detected and the *Xho*I linker from *neoX* was not transferred in a reciprocal fashion to the *neo-526* segment. We cannot, however, rule out double-reciprocal recombination between sister chromatids, since only one of the two possible chromatids was analyzed. It is also worth noting that gene conversion frequencies in mammalian cells may also depend on other parameters like the amount of homology, the size of the insertion or deletion that is to be converted, the distance separating the interacting sequences, and the site of integration in the genome. These variables will have to be analyzed systematically before a clear understanding of the mechanism of gene conversion emerges.

We are currently using analogous strategies to measure frequencies of interchromosomal gene conversion. Since site-specific integration of genes appears to be extremely rare (8, 16, 17), it would be of interest to determine whether genetic mutations can be corrected by gene conversion from segments of DNA introduced artificially elsewhere into the genome. We are also exploring whether gene conversion can be used as a mechanism to exchange genetic information between replicating extrachromosomal molecules and the chromosomes of mammalian cells. If these events are more frequent than site-specific integration, then gene conversion could be exploited to correct chromosomal mutations.

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LITERATURE CITED

1. **Baltimore, D.** 1981. Gene conversion: some implications for immunoglobulin genes. Cell **24**:592–594.
2. **Beck, E., G. Ludwig, E. A. Auerswald, B. Reiss, and H. Schaller.** 1982. Nucleotide sequence and exact localization of the neomycin phosphotransferase gene from transposon Tn*5*. Gene **19**:327–336.
3. **Gluzman, Y.** 1981. SV40-transformed simian cells support the replication of early SV40 mutants. Cell **23**:175–182.
4. **Hirt, B.** 1967. Selective extraction of polyoma DNA from infected mouse cell cultures. J. Mol. Biol. **26**:365–369.
5. **Hochtl, J., and H. G. Zachau.** 1983. A novel type of aberrant recombination in immunoglobulin genes and its implication for *V-J* joining mechanism. Nature (London) **302**:260–263.
6. **Latt, S. A.** 1974. Localization of sister chromatid exchange in human chromosomes. Science **185**:74–76.
7. **Lewis, S., N. Rosenberg, F. Alt, and D. Baltimore.** 1982. Continuing kappa-gene rearrangement in a cell line transformed by Abelson murine leukemia virus. Cell **30**:807–816.
8. **Lin, F.-L., K. Sperle, and N. Sternberg.** 1985. Recombination in mouse L cells between DNA introduced into cells and homolgous chromosomal sequences. Proc. Natl. Acad. Sci. USA **82**:1391–1395.
9. **Lin, F.-L., and N. Sternberg.** 1984. Homologous recombination between overlapping thymidine kinase gene fragments stably inserted into a mouse genome. Mol. Cell. Biol. **4**:852–861.
10. **Liskay, R. M., and J. L. Stachelek.** 1983. Evidence for intrachromosomal gene conversion in cultured mouse cells. Cell **35**:157–165.
11. **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.
12. **Luria, S. E., and M. Delbruck.** 1943. Mutations of bacteria from virus sensitivity to virus resistance. Genetics **28**:491–511.
13. **Rubnitz, J., and S. Subramani.** 1985. Rapid assay for extrachromosomal homologous recombination in monkey cells. Mol. Cell. Biol. **5**:529–537.
14. **Schimke, R. T.** 1982. Gene amplification. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
15. **Siu, G., S. P. Clark, Y. Yoshikai, M. Malissen, Y. Yanagi, E. Strauss, T. W. Mak, and L. Hood.** 1984. The human T cell antigen receptor is encoded by variable, diversity, and joining gene segments that rearrange to generate a complete V gene. Cell **37**:393–401.
16. **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.
17. **Smithies, O., R. G. Gregg, S. S. Boggs, M. A. Koralewski, and R. S. Kucherlapati.** 1985. Insertion of DNA sequences into the human chromosomal β-globin locus by homologous recombination. Nature (London) **317**:230–234.
18. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
19. **Southern, P. J., and P. Berg.** 1982. Transformation of cells to antibiotic resistance with a bacterial gene under control of the SV40 early region promoter. J. Mol. Appl. Genet. **1**:327–341.
20. **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.
21. **Wigler, M., S. Silverstein, L. S. Lee, A. Pellicer, Y. Cheng, and R. Axel.** 1977. Transfer of purified herpes thymidine kinase gene to cultured mouse cells. Cell **11**:223–232.