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Stimulation of intrachromosomal homologous recombination in mammalian cells by an inhibitor of poly(ADP-ribosylation)

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ABSTRACT

We determined the effect of 3-methoxybenzamide (3-MB), a competitive inhibitor of poly(ADP-ribose) polymerase (E.C. 2.4.2.30), on intrachromosomal homologous recombination in mouse Ltk⁻ cells. We used a cell line that contained in its genome two defective Herpes thymidine kinase (tk) genes as closely linked direct repeats. Intrachromosomal homologous recombination events were monitored by selecting for tk-positive segregants that arose during propagation of the cells and recombination rates were determined by fluctuation analysis. We found that growth of cells in the continuous presence of 2mM 3-MB increased intrachromosomal recombination between 3 and 4-fold. Growth of cells in the presence of 2mM m-anisic acid, a non-inhibitory analog of 3-MB, had no effect on intrachromosomal recombination rates. Additionally, we found that 3-MB increased both gene conversions and crossovers to similar extents, adding to the evidence that these two types of intrachromosomal rearrangements share a common pathway. These findings contrast with our previous studies [Waldman, B.C. and Waldman, A.S. (1990) Nucleic Acids Res., 18, 5981 – 5988] in which we determined that 3-MB inhibits illegitimate recombination and has no effect on extrachromosomal homologous recombination in mouse Ltk⁻ cells. An hypothesis is offered that explains the influence of 3-MB on different recombination pathways in mammalian cells in terms of the role that poly(ADP-ribosylation) plays in DNA break-repair.

INTRODUCTION

Homologous recombination in its various forms has been implicated in a variety of cellular processes including gene expression, evolution, and carcinogenesis (see 1–6 for reviews). In recent years, homologous recombination in mammalian cells has received increased attention because of the powerful tool that targeted recombination between a transfected DNA molecule and an homologous chromosomal sequence promises to bring to molecular genetics and medicine (reviewed in 1,7–9). Despite the fundamental role that homologous recombination plays in natural biological processes and the important tool that it offers, very little is known about the basic mechanisms that govern such genetic rearrangements in higher eukaryotes.

Models for extrachromosomal recombination in mammalian cells have been based on the observation that double-strand DNA breaks induce recombination near the broken sequences (1,4). There are indications that strand breakage may promote chromosomal rearrangements in mammalian cells as well. For example, genetic defects in DNA repair seem to correlate with an elevation in sister chromatid exchanges (SCE) and other chromosomal abnormalities (5,10,11).

One approach to learn about the relationship between strand breaks and intrachromosomal homologous recombination would be to measure the effect of inhibition of break-repair on the recombination process. Poly(ADP-ribosylation) is known to play an important role in DNA break-repair in higher eukaryotes, possibly by activating DNA ligase II (12,13). Potent competitive inhibitors of poly(ADP-ribose)polymerase (E.C. 2.4.2.30), the enzyme responsible for transferring ADP-ribose moities to proteins, are available. One such inhibitor is the compound 3-methoxybenzamide (3-MB) (14). It has been observed (5,10) that Chinese hamster ovary cells grown in the presence of inhibitors like 3-MB display many of the characteristics of cells that are genetically deficient in DNA break-repair, including a dramatic elevation in levels of SCE.

Treatment of a variety of cultured mammalian cells with inhibitors of poly(ADP-ribosylation) has been shown to reduce random integration (illegitmate recombination) of transfected DNA molecules into the genome (15,16). We have previously reported (16) that while treatment of mouse fibroblasts with 3-MB inhibits random integration of transfected DNA, such treatment does not effect the rate of extrachromosomal homologous recombination among the transfected molecules. These observations led to the conclusion that those two recombination pathways in mammalian cells are biochemically distinct.

The effect of inhibitors of poly(ADP-ribosylation) on intrachromosomal homologous recombination has never been determined. To further our studies on the various recombination mechanisms that operate in mammalian cells, we have examined the effect of 3-MB on *intrachromosomal* homologous recombination in cultured mouse fibroblasts. In this work we show

that, in contrast to our earlier observations made for illegitmate and extrachromosomal homologous recombination, growth of mouse fibroblasts in the continuous presence of 2mM 3-MB increased the rate of intrachromosomal homologous recombination nearly 4-fold. Both gene conversions as well as single crossovers were increased to similar extents, providing additional evidence for a mechanistic association between these two types of intrachromosomal rearrangements in mammalian cells.

MATERIALS AND METHODS

Chemicals and Enzymes

3-methoxybenzamide (3-MB) and m-anisic acid (m-AA) were purchased from Aldrich Chemical Company (Milwaukee, WI). Stock solutions of 2M 3-MB or m-AA were prepared in DMSO (Sigma Chemical Co., St. Louis, MO) and stored in aliquots at −20°C.

Restriction enzymes were purchased from New England Biolabs (Beverly, MA) or Boehringer Mannheim Biochemicals (Indianapolis, IN) and used as prescribed by the supplier.

Cell culture

The cell line 333M used in these recombination studies was obtained from R. Michael Liskay (Yale University School of Medicine) and has been described in detail by others previously (17). This cell line was derived from the thymidine kinase (tk) deficient mouse L cell line and contains a single stably integrated copy of a plasmid carrying duplicated Herpes tk gene sequences, each with an 8 bp Xho I linker insertion mutation at a different site. The linker insertions map at nucleotide positions 735 (mutant 26) and 1220 (mutant 8) of the tk gene [numbering according to Wagner et al. (18)]. The *neo* gene encoding resistance to G418 is located between the two defective tk genes.

Cells were cultured in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum (heat inactivated), MEM non-essential amino acids (GIBCO), gentamicin (50 μg/ml), and either 3-MB (2mM), m-AA (2mM), or no further additions. Cells were maintained in a humidified atmosphere of 5% CO_2, 95% air at 37°C.

Cell culture medium containing 2mM 3-MB or m-AA was prepared by prewarming the medium to 37°C followed by slow addition of the appropriate stock solution. Upon the addition of stock solution to medium, some 3-MB or m-AA would occasionally come out of solution but would redissolve upon gentle swirling. Medium containing 3-MB or m-AA was filter sterilized.

Medium containing hypoxanthine/aminopterin/thymidine (HAT) (19) was prepared using HAT medium suppplement (Sigma Chemical Co., St. Louis, MO.)

Determination of intrachromosomal homologous recombination rates

Fluctuation analyses were performed as described previously (17). Briefly, 10 independent subclones were used in each rate determination. Starting from fewer than 50 cells per subclone, each subclone was grown in the continuous presence of 2mM 3-MB, 2mM m-AA or no drug supplement until greater than 2×10^6 cells were obtained per subclone. Each subclone was then plated into HAT medium (19) to select for tk-positive segregants. The appropriate drug supplement (2mM 3-MB, 2mM m-AA, or no drug) was present for the duration of HAT selection (14 days), after which time colonies were stained and counted.

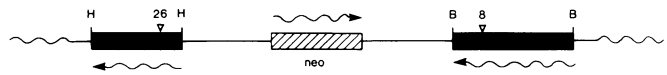

Figure 1. Organization of the recombination substrate contained in the genome of cell line 333M. Line 333M contains a single copy of the depicted construct. The construct is based on the pSV2neo vector, represented by the thin line, and contains a *neo* gene (▨) as well as two fragments of Herpes DNA (■) harboring defective tk genes. One tk gene is contained within a DNA fragment flanked by Hind III sites (H) while the other is flanked by Bam HI sites (B). The tk genes are rendered nonfunctional by two different 8 bp Xho I linker insertions shown as '26' and '8.' The Hind III fragment (2 kb) is divided into 1.5 kb and 0.5 kb intervals by mutation #26 while the Bam HI fragment (2.5 kb) is divided into 1.5 kb and 1.0 kb intervals by mutation #8. (Drawing is not to scale.) The distance between the tk genes is approximately 4 kb. The direction of transcription of each gene is indicated by a wavy arrow. See Materials and Methods for more details.

The numbers of HAT^r colonies obtained for the 10 subclones were used to calculate the rate of recombination in terms of events/cell/generation by the method of Luria and Delbruck (20) as described (21).

Southern hybridization analysis

DNA isolation and Southern blotting analysis were accomplished as previously described (22). A probe specific for the Herpes tk gene was used in all analyses.

RESULTS

Experimental Strategy

As part of a model system for the study of intrachromosomal homologous recombination in cultured mammalian cells, the cell line 333M had been constructed and described previously (17). This cell line is derived from the mouse Ltk^- fibroblast line and contains a single copy of the construct illustrated in figure 1 stably integrated in its genome. This construct contains direct repeats of two defective Herpes simplex virus type one tk genes flanking a *neo* marker gene and separated by approximately 4 kb of vector sequences (figure 1). The two tk genes are rendered nonfunctional by 8bp Xho I linker insertions that create frame shift mutations and place a unique Xho I site in each tk sequence. The rate of reversion of these mutations is less than 1 reversion per 10^8 mitotic divisions (17). Recombination events between the two defective tk sequences that result in the reconstruction of a functional tk gene can be monitored by selecting for HAT^r segregants that arise during propagation of cell line 333M.

We were interested in using line 333M to determine what effect inhibition of poly(ADP-ribosylation) has on intrachromosomal homologous recombination in mammalian cells. To inhibit poly(ADP-ribosylation) we cultured line 333M in the continuous presence of 3-MB, a competitive inhibitor of poly(ADP-ribose)polymerase (14). In control experiments we used m-AA, a noninhibitory analog of 3-MB (14). A concentration of 2mM 3-MB (or 2mM m-AA) was chosen to be consistent with our earlier studies (16) on the effect of 3-MB on illegitimate and extrachromosomal homologous recombination in mouse fibroblasts as well as studies by others (15) on the effect of such inhibitors on illegitimate recombination in a variety of mammalian cells.

We had previously shown that the addition of 2mM 3-MB to growth medium increased the doubling time of mouse Ltk^- cells marginally, from 16.5 to 18 hours (16). We had also determined that the plating efficiency of Ltk^- cells in the presence of 2mM

Table 1. Effect of Growth in 2mM 3-MB or 2mM m-AA on the Rate of Intrachromosomal Homologous Recombination

Expt	Medium Supplement	Fluctuation Test Results[a]					Rate of Recombination[b] ($\times 10^6$)
	None	243/6.0	72/6.0	30/6.0	25/6.0	37/6.0	2.3
		37/6.0	24/6.0	19/6.0	88/6.0	117/6.0	
1	m-AA	31/6.0	57/6.0	20/6.0	27/6.0	81/6.0	1.4
		16/6.0	21/6.0	33/6.0	60/6.0	22/6.0	
	3-MB	36/2.3	329/2.1	25/2.2	24/2.0	30/2.2	8.9
		112/2.4	31/2.0	60/2.4	26/2.0	337/2.3	
	None	119/6.0	20/6.1	15/5.9	60/6.0	14/6.0	1.6
		17/6.0	26/6.1	121/5.8	19/6.0	28/6.1	
2	m-AA	88/6.1	43/6.0	53/6.0	27/6.0	39/6.1	2.1
		48/6.0	50/5.9	215/5.9	29/6.0	25/5.9	
	3-MB	73/2.6	75/2.3	35/2.0	74/2.0	18/1.2	6.0
		4000/2.3[c]	150/2.3	31/2.5	35/1.6	30/1.9	(33.0)
3	3-MB	204/3.3	36/3.2	25/2.5	83/3.1	46/2.6	6.4
		237/3.3	129/3.3	37/2.7	155/3.0	28/2.4	

[a] Data for each subclone is displayed in the form x/y where x=number of HAT^r colonies recovered and y=number of cells, in millions, plated into HAT medium. For fluctuation analysis in the presence of 2mM 3-MB, cell numbers were corrected for the reduced relative plating efficiency (55%) in 2mM 3-MB (16).

[b] Number of recombination events/cell/generation.

[c] This subclone was judged to be a 'jackpot.' A recombinant apparently arose early in the growth of this subclone resulting in an aberrantly large number of HAT^r colonies. Because this subclone is not representative, we have omitted it in calculating the recombination rate. Rate including this jackpot is shown in parenthesis.

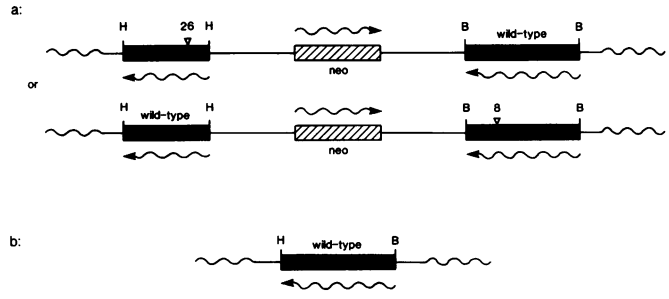

Figure 2. Two types of recombination events that occur in line 333M. Illustrated are gene conversions (panel a) and a single crossover (panel b) that result in the reconstruction of a functional tk gene. The uppermost diagram depicts the conversion to wild-type of the mutant tk gene contained on the Bam HI fragment. The second line depicts a conversion of the tk gene contained on the Hind III fragment. In gene conversions, one Xho I linker is removed and no other change is made at the locus. Gene conversion is indicated in Southern analysis if either the 2.0 kb Hind III fragment or the 2.5 kb Bam HI fragment is resistant to Xho I cleavage while the other fragment can be cleaved into the appropriate fragments described in the legend to figure 1. The *neo* gene is retained in conversion events. As shown in panel b, a single crossover (either intrachromatid or unequal sister chromatid exchange) results in loss of sequences between the tk genes and subsequently the cell becomes sensitive to G418. A wild-type tk gene contained on a 2.5 kb Hind III-Bam HI fragment is produced. No other tk sequence remains at the locus. Symbols and notations are as in Figure 1.

3-MB was 55% of that observed in the absence of drug (16). Plating efficiency in the presence of 2mM m-AA was >95% that in the absence of drug (16).

Line 333M was subcloned, propagated and subjected to fluctuation analyses in the presence of 2mM 3-MB, 2mM m-AA or no drug supplement to determine the rate of appearance of tk-positive segregants under the three different conditions. Further determinations, as described below, of the percentage of gene conversions (nonreciprocal exchanges) versus single crossovers (reciprocal exchanges) were made to learn about the qualitative effects of inhibition of poly(ADP-ribosylation) on recombination.

3-MB increases the overall rate of intrachromosomal homologous recombination

Fluctuation analyses were performed on cell line 333M grown in the absence of any drug or in the presence of 2mM 3-MB or 2mM-AA and the results of these analyses are summarized in Table 1. As shown, the recombination rates in the absence of drug or in the presence of 2mM m-AA were nearly equal. The average recombination rate for cells in the absence of drug was 2.0×10^{-6} while the average rate for cells grown with 2mM m-AA was 1.8×10^{-6} recombination events per cell per generation. These rates were consistent with previously published recombination rates for line 333M propagated in the absence of any drug (17). In contrast, based on three independent determinations, the average rate of appearance of tk-positive segregants for line 333M grown in the presence of 2mM 3-MB was 7.1×10^{-6} events per cell per generation (Table 1), nearly 4-fold greater than that observed in either the absence of drug or in the presence of 2mM m-AA. This calculation of average rate excludes the 'jackpot' subclone in experiment 2 (see footnote to Table 1). In fact, if this jackpot is included, the average rate becomes 1.6×10^{-5}, over 8-fold greater than the rates for the control experiments. Additionally, the absolute number of HAT^r colonies recovered for cells grown with 2mM 3-MB exceeded the absolute number of colonies recovered in parallel analyses done in either the absence of drug or the presence of m-AA.

3-MB increases the rates of both gene conversions and single crossovers

It had been reported previously (17) that greater than 75% of spontaneous intrachromosomal recombination events between the defective Herpes tk sequences in 333M are gene conversion events (nonreciprocal exchanges) rather than single crossovers. There is evidence that gene conversions and crossovers in mammalian cells are mechanistically associated (23,24) and represent the outcomes of alternative modes of resolving recombination intermediates generated by a common initiation mechanism (25). We wanted to determine if propagation of line 333M in the presence of 2mM 3-MB altered the ratio of conversions to crossovers in addition to increasing the overall intrachromosomal recombination rate. An alteration in the relative recoveries of gene conversions versus crossovers would suggest that the presence of 3-MB influenced a late step in the recombination pathway or alternatively that conversions and crossovers involve biochemically distinct pathways.

The presence of the *neo* marker gene between the defective tk sequences allowed a simple genetic screen to determine whether a HAT^r segregant arose due to gene conversion or a reciprocal exchange. As illustrated in figure 2, gene conversions result in retention of the *neo* marker gene between the tk sequences in the chromosome while single crossovers (either intrachromatid or unequal sister chromatid exchanges) result in loss of the *neo* marker. The presence or absence of the *neo* marker can be determined by a cell's ability to grow in the presence of G418.

We tested HAT^r clones that arose in each of the fluctuation tests presented in Table 1 for their abilities to grow in G418.

Table 2. Effect of 3-MB on Relative Rates of Gene Conversions Versus Crossovers

Drug Supplement	Expt.	No. of HAT^r Clones Tested[a]	No. of G418^r clones	Percentage G418^r
None	1	5	4	80
	2	10	10	100
	Total	15	14	93
m-AA	1	5	4	80
	2	10	9	90
	Total	15	13	87
3-MB	1	4	3	75
	2	18	11	61
	3	17	15	88
	Total	39	29	74

[a] For each clone tested, samples of approximately 5,000 cells were plated into two wells of a 24-well dish. One well contained DMEM and the other contained DMEM plus G418 (400 μg per ml). After 10 days of incubation, cell growth in the two wells were compared.

The results are listed in Table 2. As shown, 93%, 87%, and 74% of the HAT^r clones recovered from cells grown with no drug supplement, 2mM m-AA, or 2mM 3-MB, respectively, retained the *neo* gene. Based on the data presented in Table 2 and the previous report that 79% of the events recovered using line 333M are gene conversions (17), we concluded that there was no appreciable difference in the percentage of HAT^r recombinants that retained the *neo* gene whether cells were grown with no drug, m-AA, or 3-MB. It was clear that both gene conversions as well as crossovers were stimulated by 3-MB. Had gene conversions been induced selectively, we would have expected the percentage of conversions among cells grown with 3-MB to be substantially greater than that for cells grown with m-AA or no drug. Similarly, had only crossovers been induced, a majority of the recovered events would have to be crossovers in order to account for the greater than 3-fold increase in the overall recombination rate.

Southern analysis of recombinants obtained in the presence of 3-MB

Genomic DNA samples isolated from HAT^r clones obtained in fluctuation tests performed in the presence of 2mM 3-MB were subjected to Southern analysis. Several representative samples are displayed on the blot shown in figure 3. Southern blotting analysis confirmed that the tk-positive segregants had each undergone an intrachromosomal homologous recombination event that reconstructed a functional tk gene. The blotting analysis indicated that recombinants that retained resistance to G418 had undergone an apparent gene conversion that corrected one of the two defective Herpes tk genes (see figure 3, lanes 1 through 4). Both of the defective tk genes in 333M were capable of serving as either donor or recipient in gene conversions. For example, the HAT^r clone examined in lanes 1 and 2 of figure 3 had undergone a correction of mutant tk gene #26 (the tk gene contained on a 2.0 kb Hind III fragment) whereas the clone displayed in lanes 3 and 4 had undergone a correction of mutant tk gene #8 (the tk gene contained on a 2.5 kb Bam HI fragment). The Southern analysis also confirmed that recombinants that had lost resistance to G418 had apparently undergone a single crossover between the tk genes. The crossovers resulted in the loss of sequences between the two tk genes as well as the formation of a tk gene flanked by Hind III and Bam HI sites (see figure 3, lanes 5 through 10). No unexpected or unusual rearrangements were observed.

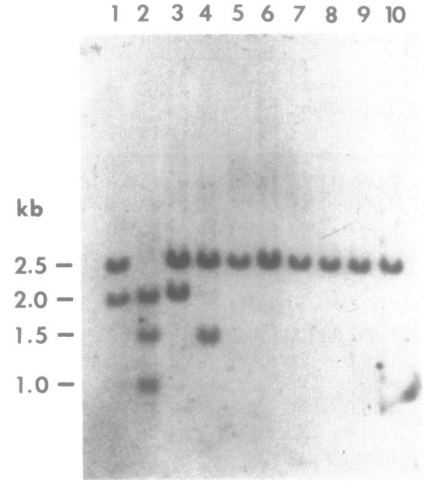

Figure 3. Southern analysis of recombinants recovered in the presence of 2mM 3-MB. DNA was isolated from HAT^r colonies and 10 μg aliquots were subjected to Southern blotting analysis. The analysis of five representative HAT^r colonies is displayed. Pairwise lanes (e.g., lanes 1 and 2) contain DNA from a single HAT^r colony digested with Bam HI plus Hind III (odd lanes) or Bam HI plus Hind III plus Xho I (even lanes). The blot was hybridized with a probe specific for Herpes tk sequences. The two HAT^r colonies represented in lanes 1–4 maintained resistance to G418. The colony displayed in lanes 1 and 2 had undergone an apparent conversion to wild-type of the tk gene contained on the 2.0 kb Hind III fragment (i.e. mutant #26, see figure 2). In lane 1, both the 2.5 kb Bam HI fragment as well as the 2.0 kb Hind III fragment are exhibited. In lane 2, the 2.5 kb fragment is cleaved with Xho I into the predicted 1.5 and 1.0 kb fragments, indicative of the presence of Xho I linker insertion mutation #8. In contrast the 2.0 kb fragment is resistant to cleavage with Xho I, indicative of correction of Xho I linker insertion mutation #26. Similarly, it was determined that the colony displayed in lanes 3 and 4 had undergone a conversion of the tk gene contained on the 2.5 kb Bam HI fragment (i.e. mutant #8, figure 2). The three HAT^r colonies represented in lanes 5–10 were sensitive to G418. As expected, each colony displayed a single 2.5 kb fragment upon digestion with Bam HI plus Hind III and the fragment was resistant to digestion with Xho I. This hybridization pattern indicated that these colonies had undergone a single crossover (see figure 2).

DISCUSSION

We have shown that propagation of mouse fibroblasts in the continuous presence of 2mM 3-MB, a competitive inhibitor of poly(ADP-ribose)polymerase, results in a nearly four-fold increase in the rate of intrachromosomal homologous recombination. Although we used 3-MB to inhibit poly(ADP-ribosylation),it is possible that a secondary effect of 3-MB unrelated to inhibition of poly(ADP-ribosylation) was responsible for altering the rate of homologous recombination. However, the fact that the non-inhibitory analog m-AA did not have any effect on intrachromosomal recombination rates makes it more likely that the influence of 3-MB was due to inhibition of poly(ADP-ribose)polymerase rather than some other metabolic effect of 3-MB. M-AA is structurally very similar to 3-MB and the most likely metabolic fate of 3-MB would be deamidation to m-AA.

How may inhibition of poly(ADP-ribosylation) elevate the rate of intrachromosomal homologous recombination? One possibility is that homologous recombination involves a protein whose activity is attenuated by poly(ADP-ribosylation). Alternatively, recombination might be influenced by the effect of 3-MB on

chromatin or DNA structure. It is possible, for example, that an alteration in the degree of chromatin condensation brought about by a decreased level of ADP-ribosylation of histone proteins (12) might influence chromosomal rearrangements.

Perhaps the effect of 3-MB on the intrachromosomal recombination rate can be interpreted in terms of DNA strand breaks. It has been reported that poly(ADP-ribose)polymerase activity from a variety of mammalian cells, including mouse L cells, is induced by and *absolutely requires* free ends of double-stranded DNA fragments (12,26). Poly(ADP-ribosylation) is known to play a role in DNA break-repair (12), possibly by activation of DNA ligase II (13), although a variety of mechanisms have been proposed (see 12). There are many reports that indicate that appropriately placed DNA strand breaks stimulate extrachromosomal homologous recombination in mammalian cells (1,4). It is therefore not unreasonable to propose that 3-MB stimulates intrachromosomal homologous recombination by effectively increasing the availability of chromosomal breaks by increasing their lifespan.

It is instructive to compare the effects of 3-MB on illegitimate recombination versus extrachromosomal or intrachromosomal homologous recombination in mouse fibroblasts. We had previously demonstrated (16) that treatment of cells with 3-MB during transfection inhibits random genomic integration (illegitimate recombination) of transfected DNA molecules but has no discernible effect on extrachromosomal homologous recombination among the transfected molecules. The different effects that 3-MB has on different types of genetic rearrangements may be a reflection of the rate-limiting step(s) of each particular process. For example, the rate-limiting step of illegitimate recombination may be the sealing of strand breaks, consistent with the idea that random integration is essentially a DNA ligation process (reviewed in 27). In contrast, the rate-limiting step of intrachromosomal homologous recombination might be the formation or availability of DNA breaks. DNA break availability might not be rate-limiting for homologous recombination among transfected molecules even in the absence of 3-MB since a great deal of damage, including strand breakage, is known to be inflicted upon transfected DNA (1). It is also possible that the different effects of 3-MB on extra-versus intrachromosomal homologous recombination is a reflection of qualitatively different homologous recombination mechanisms operating within the mammalian cell. Consistent with this possibility is our previous observation of differential sensitivities of the two types of homologous recombination pathways to sequence heterologies (22).

It has been reported (28) that transfected oncogenes are lost from the genome of NIH 3T3 cells when the cells are cultured for several weeks in the continuous presence of any of a number of inhibitors of poly(ADP-ribose)polymerase. The precise mechanism for such genomic deletions is presently unclear, but the observations provide further evidence that poly(ADP-ribosylation) plays a role in the maintenance of genomic stability in mammals.

We cannot formally distinguish between 3-MB increasing the rate of *initiation* versus the rate of *resolution* of recombination. We prefer the notion that 3-MB increases initiation of recombination. We observed no significant effect on the relative rates of gene conversions versus crossovers so we obtained no evidence that the resolution process was affected by the presence of 3-MB in the growth medium. A parallel increase in both gene conversions and crossovers is accommodated economically by the assumption that 3-MB stimulates recombination initiation and the notion that conversions and crossovers are alternate modes of resolution of a single recombination initiation pathway (25). The observation that 3-MB induces both gene conversions and single crossovers thus adds to the evidence (23,24) that gene conversions and crossovers are mechanistically associated in mammalian cells.

Finally, the demonstration that homologous recombination at a chromosomal locus can be increased by the use of a compound that inhibits random integration of transfected DNA into the genome (15,16) suggests that illegitmate and homologous recombination are in a sense competing pathways of nucleic acid metabolism in mammalian cells. To theorize that the absolute frequency of gene targeting in mammalian cells can be elevated by blocking random integration may therefore be fundamentally correct.

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