Invited paper: XV International Congress of Genetics, Satellite Symposium on Mutagenesis: Basic and Applied

CONF-831258--1

DE84 004428

QUANTITATIVE AND MOLECULAR ANALYSES OF MUTATION IN A pSV2gpt TRANSFORMED CHO CELL LINE

Leon F. Stankowski, Jr.,[1] Kenneth R. Tindall,[1,3] and Abraham W. Hsie[2]

The University of Tennessee-Oak Ridge Graduate School of Biomedical Sciences[1], and Biology Division, Oak Ridge National Laboratory[2], Oak Ridge, TN 37831

[3]Present Address: Department of Molecular Biophysics and Biochemistry
Yale University
P. O. Box 6666
New Haven, CT 06511

Research sponsored by Office of Health and Environmental Research, U. S. Department of Energy under contract W-7405-eng-26 with the Union Carbide Corporation.

ABSTRACT

Following DNA-mediated gene transfer we have isolated a cell line useful for studying gene mutation at the molecular level. This line, AS52, derived from a hypoxanthine-guanine phosphoribosyl transferase (HGPRT) deficient Chinese hamster ovary (CHO) cell line, carries a single copy of the *E. coli* xanthine-guanine phosphoribosyl transferase (XGPRT) gene (*gpt*) and exhibits a spontaneous mutant frequency of 20 TG^r mutants/10^6 clonable cells. As with $HGPRT^-$ mutants, $XGPRT^-$ mutants can be selected in 6-thioguanine. AS52 ($XGPRT^+$) and wild type CHO ($HGPRT^+$) cells exhibit almost identical cytotoxic responses to various agents. We observed significant differences in mutation induction by UV light and ethyl methanesulfonate (EMS). Ratios of $XGPRT^-$ to $HGPRT^-$ mutants induced per unit dose (J/m^2 for UV light and μg/ml for EMS) are 1.4 and 0.70, respectively. Preliminary Southern blot hybridization analysis has been performed on 30 $XGPRT^-$ AS52 mutants. A majority of spontaneous mutants have deletions ranging in size from 1-4 kilobases (9/19) to complete loss of *gpt* sequences (4/19); the remainder have no detectable (5/19) or only minor (1/19) alterations. 5/5 UV-induced and 5/6 EMS-induced mutants do not show a detectable change. Similar analyses are underway for mutations induced by X-irradiation and ICR 191 treatment.

INTRODUCTION

While there is no doubt that mammalian cells undergo mutation and that specific locus mutation induction can be quantified (1), there are no existing systems using mammalian cells which provide for the direct analysis of an altered DNA sequence in these mutants. The best mammalian cell systems used to study mutation have not examined the DNA directly, but instead have extrapolated from an altered protein primary sequence, immunological variants, altered enzyme kinetics, or genetic reversion (2-5). Developments in recombinant DNA techniques have provided methods applicable to the molecular analysis of mutations in mammalian cells.

These techniques have met with considerable success in the analysis of mutations at selectable loci in bacteriophage, bacterial and yeast systems (6). Advances in our understanding of the molecular basis of mutation in these systems are due largely to the availability of various selectable genetic markers, many of which can be cloned and isolated directly in either the parental or mutant form. Fine structure mapping followed by inferred or direct sequence analysis of the mutation site has been one widely used approach (7-9).

These techniques, however, are not as readily applicable to studies of gene mutation in mammalian cells. There are relatively few loci in mammalian cells in which a similar pattern of mutant and revertant selection schemes can be applied. Isolation of specific genes for mutational analysis is compounded by the presence of intervening sequences, the quantities of DNA in mammalian cells, and the difficulty of devising rapid isolation schemes for the DNA sequence analysis of cloned mutant loci.

Nevertheless, mutations have been observed at the dihydrofolate reductase (*dhfr*), adenine phosphoribosyl transferase (*aprt*), and hypoxanthine-guanine phosphoribosyl transferase (*hgprt*) loci in Chinese hamster cells using Southern blot hybridization of parental and mutant genomic DNA sequences (10-12).

A limitation of these approaches is that only major genetic alterations such as deletions and rearrangements, or fortuitous minor alterations within a restriction enzyme site, are detectable. To date, rapid methods by which point mutations in mammalian loci can be localized and sequenced have not been devised.

DNA-mediated gene transfer techniques have the potential to be useful in the analysis of gene mutation in mammalian cells; yet few studies in this area have been initiated. The herpes simplex virus thymidine kinase (*tk*) gene in TK-deficient transformed mouse L cells has been used to study the ultimate fate and organization of transforming DNA sequences (13). Several investigations (13-16) have identified specific changes in integrated *tk* sequences associated with cells selected to express the TK^- phenotype. Recently, analysis of mutation induction at the *E. coli gpt* locus in pSV2*gpt* transformed Chinese hamster cells has been reported (17, 18). In these studies alterations in the *gpt* gene were detected in cells selected to express the gpt^- phenotype. However, relatively few other dominant selectable markers or sufficiently defined mammalian recipient cell lines are available for similar studies.

We have developed a system to study mutations which affect *gpt* gene expression in $HGPRT^-$ Chinese hamster ovary (CHO) cells which have been transformed by the plasmid vector pSV2*gpt* (19). The pSV2*gpt* plasmid car-

ries the E. coli gpt gene and through the use of the SV40 early promoter expresses the bacterial enzyme xanthine-guanine phosphoribosyl transferase (XGPRT) in mammalian cells (Fig. 1). One isolated CHO transformant, designated AS52, carries a single copy of the gpt gene stably integrated into the high molecular weight DNA (Fig. 2). Bacterial XGPRT and mammalian HGPRT are functionally analogous purine salvage pathway enzymes. However, under physiological conditions XGPRT catalyzes the formation of xanthine 5'-monophosphate (XMP) from xanthine while HGPRT does not. Selection protocols utilizing the purine analog 6-thioguanine (TG) derived for the study of mutation at the hgprt locus in CHO cells (20) can be used to select for mutations at the gpt gene in the pSV2gpt transformed CHO line.

In this study, we show that mutation induction at the gpt locus in pSV2gpt transformed CHO cells can be quantified, and compared to mutation induction at the hgprt locus in parental CHO cells. All 30 TG^r derivatives of the AS52 line examined exhibit no detectable XGPRT activity. In 15 of these clones, loss of XGPRT activity correlates with alterations at the gpt locus detected by Southern blot analysis.

METHODS

Cell culture. The parental CHO-K1-BH4 line (*hgprt*$^+$), the AS52 line (*hgprt*$^-$, *gpt*$^+$), and culture conditions for each have previously been described (18, 20).

Mutagenesis experiments. Mutagen treatment, determination of cytotoxicity and mutant selection were performed as in the standard CHO/HGPRT assay as previously described (21).

Statistical analysis of mutation data. Least squares linear regression analysis was used to fit a line to the data presented in Table 1. The induced TG^r mutant frequency is equal to the slope of the line fitted to these data. Paired data points were compared to determine if significant differences (student's t-test) occurred in mutation induction at each locus for each mutagen.

Analysis of XGPRT activity. Prior to preparation of cell free extracts, TG^r clones derived from the AS52 line were maintained in non-selective medium for at least 10 days. Extracts were prepared and analyzed for enzymatic activity using a filter binding assay as previously described (5), except using [^{14}C]-xanthine as substrate.

Southern blot analysis. Genomic DNA from all cell lines was isolated and digested with the appropriate restriction enzyme, transferred to nitrocellulose filters and hybridized against a [^{32}P]-nick-translated pSV2*gpt* probe. Hybridization was allowed to continue for 24-40 hours at 42°C. Filters were then washed for 3 hours with 3 to 5 changes in 2X SSC-0.1%

SDS. Air dried filters were then exposed to Kodak XAR-5 film with intensifying screens at -70°C for 1 to 7 days.

RESULTS

Mutation induced at the gpt and hgprt loci. A comparison of relative survival and mutation induction at the gpt and hgprt loci in the AS52 and CHO-K1-BH4 cells, respectively, following UV light-irradiation or ethyl methanesulfonate (EMS) treatment is shown in Table 1. Note that the relative survival of both cell lines is quite similar in response to each agent. Data presented in Table 1 indicate a dose-dependent increase in UV light- and EMS-induced mutation at the gpt locus in AS52 cells. Mutation induction at the gpt locus, however, is significantly different ($p < .005$) from those seen for the hgprt locus in the CHO-K1-BH4 line. Based upon data from these and additional experiments AS52 cells exhibit an average spontaneous mutant frequency of 20 TG^r mutants/10^6 clonable cells.

Linear regression analysis was used to calculate and compare the induced TG^r mutant frequency for each cell type in response to each agent. The results are presented in Table 2. UV light induces 74 and 54 TG^r mutants/10^6 clonable cells/J/m^2 at the gpt and hgprt loci, respectively. EMS induces 0.82 and 1.2 TG^r mutants/10^6 clonable cells/ug/ml of EMS at the gpt and hgprt loci, respectively. Ratios of $XGPRT^-$ to $HGPRT^-$ mutants induced per unit dose are 1.4 and 0.70 for UV-light and EMS, respectively.

Analysis of XGPRT activity. Thirty independently isolated TG^r derivatives of the AS52 cell line were analyzed for XGPRT activity using an *in vitro* filter binding assay as previously described (5). None of these TG^r clones (19 spontaneous, 5 UV-induced, and 6 EMS-induced mutants) exhibited any detectable enzymatic activity [<0.2 nmole XMP formed/min/mg protein vs. 20 nmole XMP formed/min/mg protein in the parental AS52 (gpt^+) cell line].

Southern blot analysis. The same 30 TG^r derivatives of AS52 were analyzed by Southern blot hybridization against a [^{32}P]-nick translated pSV2gpt probe. All clones were analyzed following digestion of genomic DNA with the restriction enzymes XbaI or HindIII (data not presented). Selected clones were further analyzed using additional single and/or double enzyme digestions. Results are presented in Figure 3 and Table 3. Alterations were observed in the Southern blot hybridization pattern of 15 of the 30 TG^r clones. A majority of the spontaneous mutants (Figure 3) have deletions ranging in size from 1-4 kilobases (9/19) to complete loss of pSV2gpt-hybridizable sequences (4/19); the remainder have no detectable (5/19), or only minor (1/19) alterations. The lone spontaneous TG^r clone with only a minor alteration has an as yet undefined mutation (point mutation or small deletion) which results in loss of the PvuII site immediately preceding the SV40 promoter sequences. 5/5 UV-induced and 5/6 EMS-induced mutants do not exhibit a major alteration; the remaining EMS-induced mutant (1/6) contains no sequences that will hybridize to pSV2gpt.

DISCUSSION

We have used the AS52 cell line, which carries a single functional copy of the *E. coli gpt* structural gene stably integrated into the genomic DNA, for quantitative and molecular analyses of mutation in mammalian cells. This cell line exhibits a spontaneous TG^r mutant frequency of approximately 20 TG^r mutants/10^6 clonable cells and shows a dose-dependent induction of TG^r mutants following UV-irradiation or EMS exposure. Thirty TG^r mutants examined exhibit no detectable XGPRT activity in an *in vitro* filter binding assay. Alteration of the pSV2*gpt* sequences was detected by Southern blot hybridization in 15 of these mutants.

While the cytotoxic effects of UV-irradiation or EMS are similar in the AS52 and CHO-K1-BH4 cells, mutation induction at the *gpt* and *hgprt* loci are quite different. When compared on a per unit dose basis, the ratios of $XGPRT^-$ to $HGPRT^-$ mutants are 1.4 and 0.70 for UV and EMS, respectively. Preliminary experiments using X-rays or ICR-191 indicate the same type of pattern, i.e., similar cytotoxic responses, but significantly different levels of mutation induction. The $XGPRT^-$ to $HGPRT^-$ mutant ratio is greater than 1.0 for treatment with X-ray or ICR-191. The basis of the difference in the slopes of the mutation induction curves of UV light- or X-irradiated or EMS or ICR-191 treated AS52 and CHO-K1-BH4 cells has yet to be resolved. Differences in the slopes of the induced mutation curves may reflect differences in target size or DNA sequence between the *gpt* and *hgprt* genes.

A number of TG^r AS52 colonies were cloned and assayed for XGPRT activity using *in vitro* enzyme activity assays. No XGPRT activity was detectable in any of the 30 TG^r lines assayed. This observation is consistent with the notion that TG^r colonies arising either spontaneously or

following mutagen treatment of the AS52 line are the result of mutation at the gpt locus, and are selectable as a result of the stringent TG selection system previously described for the isolation of mutants at the hgprt locus in mammalian cells (21).

Alterations in the Southern blot hybridization patterns were detected in 15 of the 30 TG^r clones examined in this study. Nineteen of these clones arose spontaneously, 5 clones were isolated following UV light-irradiation, and 6 following EMS treatment. These latter 11 clones are labeled "UV- or EMS-induced" TG^r mutants because they were isolated following mutagen treatments which resulted in an induced mutant frequency that was at least 10 times greater than the background spontaneous mutant frequency. Thus, it is most likely that these TG^r mutants were induced by UV light or EMS.

14/19 spontaneous TG^r mutants contain detectable alterations in the pSV2gpt sequences. One clone has an apparent point mutation or very small deletion, while the remainder contain deletions ranging in size from approximately 1 kb to complete loss of pSV2gpt-hybridizable sequences. Due to the limited number of restriction enzyme digestions used, precise localizations of all these deletions cannot be made. However, all detectable alterations involve at least a portion of the gpt structural sequences, the SV40 regulatory sequences, or both. Several of the deletion mutations were found to extend into non-plasmid, genomic sequences. Only one of the induced mutants (isolated after EMS treatment) exhibits any gross, detectable alteration; it no longer contains any hybridizable sequences. Since a large proportion of spontaneous mutants were found to contain deletions, and since it was reported that EMS did not induce large deletions in

Chinese hamster cells (11), this clone may represent a spontaneously arising TG^r clone which happened to be isolated following mutagen treatment. The remaining induced mutants contain no gross alterations.

Even from the limited number of TG^r mutants analyzed in this study, it is apparent that spontaneous and UV light- or EMS-induced mutants arise by different mechanisms. Almost 70% (13/19) of the spontaneously arising TG^r mutants contain gross deletions of approximately 1 kb or more. This high proportion of spontaneous deletion mutations at the gpt locus is consistent with previous observations of spontaneous deletion mutations affecting the E. coli lacI gene after transformation into mammalian cells (22). However, studies of spontaneous mutation at the hgprt locus in Chinese hamster V79 cells indicate that the frequency of deletion events is relatively low (12). Whether this reflects an intrinsic characteristic of the bacterial gpt and lacI genes, or is due to its presence and/or location within foreign DNA sequences, remains to be studied. In contrast, no UV-induced mutants (0/5) and only one EMS-induced mutant (1/6) have any detectable alterations in the pSV2gpt sequences. This is consistent with the notion that both UV-light and EMS induced predominantly missense mutations (6).

While only deletion mutations and one undefined minor alteration were detected in these studies, further efforts should provide for the direct analysis of mutant DNA sequences in this system, as well. The gpt gene (1.0 kb) and related plasmid sequences at the insertion site total approximately 4.0 kb (manuscript submitted) which makes the subcloning of mutant DNA sequences from within the AS52 genome relatively straightforward. Once isolated, the DNA fragment containing the mutation can be used for DNA

sequence analysis.

This kind of approach to mutation studies allows a means by which mutagen specific changes can be correlated with specific types of DNA base sequence alterations. Some investigators have been successful in analyzing mutagen specificity *in vitro* and in some viral and bacterial systems using this type of correlative approach. Of course, a limitation to these kinds of studies is that specific mutations observed can only be correlated to mutagen activity following the analysis of a large number of mutants, since any individual mutation analyzed has a small probability of being spontaneous in origin as discussed earlier. Nevertheless, the possibility of analyzing point mutations within a selectable locus defined by a fairly small fragment of DNA, as exists in the AS52 cell line, is advantageous for use in the molecular analysis of mutation in mammalian cells.

ACKNOWLEDGEMENTS

We thank Paul Berg and Richard Mulligan for supplying the pSV2*gpt* plasmid, R. Julian Preston and Richard Machanoff for reviewing this manuscript and for suggestions to its improvement, and Faye Young and Nette Crowe for their assistance in the preparation of this manuscript. K.R.T. and L.F.S. Jr. were supported by an NIGMS predoctoral training grant in genetics (NIH Grant GM 7438). Research sponsored by the Office of Health and Environmental Research, U.S. Department of Energy under contract W-7405-eng-26 with the Union Carbide Corporation.

REFERENCES

1. Hsie, A. W., J. P. O'Neill and V. K. McElheny (eds.) (1979) Mammalian Cell Mutagenesis. Banbury Report #2. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
2. Caskey, C. T. and G. D. Kruh (1979) The HPRT locus. Cell 16:1-9.
3. Milman, G., S. W. Krauss and A. S. Olsen (1977) Tryptic peptide analysis of normal and mutant forms of HPRT from HeLa cells. Proc Natl. Acad. Sci. USA 74: 926-930.
4. Fenwick, R. G., Jr., T. H. Sawyer, G. D. Kruh, K. H. Astrin and C. T. Caskey (1977) Forward and reverse mutations affecting the kinetics and apparent molecular weight of mammalian HGPRT. Cell 12: 383-391.
5. Fuscoe, J. C., J. P. O'Neill, R. Machanoff and A. W. Hsie (1982) Quantification and analysis of reverse mutations at the hgprt locus in Chinese hamster ovary cells. Mutat. Res. 96: 15-30.
6. Lemontt, J. F. and W. M. Generoso (eds) (1982) Molecular and cellular Mechanisms of Mutagenesis (Proceedings), 388 pp., Plenum Press, NY.
7. Duncan, B. K. and J. H. Miller (1980) Mutagenic deamination of cytosine residues in DNA. Nature 287: 560-561.
8. Eisenstadt, E., A. J. Warren, J. Porter, D. Atkinds and J. H. Miller (1982) Carcinogenic epoxides of benzo(a)pyrene and cyclopenta(cd)-pyrene induce base substitutions via specific transversion. Proc. Natl. Acad. Sci. USA 79: 1945-1949.
9. Skopek, T. R. and F. Hutchinson (1982) DNA base sequence changes induced by bromouracil mutagenesis of lambda phage. J. Mol. Biol. 159: 19-33.
10. Graf, L. H., Jr. and L. A. Chasin (1982) Direct demonstration of

genetic alterations at the dihydrofolate reductase locus after gamma irradiation. Mol. Cell. Biol. 2: 93-96.

11. Meuth, M. and J. E. Arrand (1982) Alterations of gene structure in ethyl methanesulfonate induced mutants of mammalian cells. Mol. Cell. Biol. 2: 1459-1462.

12. Fuscoe, J. C., R. G. Fenwick, Jr., D. H. Ledbetter and C. T. Caskey (1983) Deletion and amplification of the HGPRT locus in Chinese hamster cells. Mol. Cell. Biol. 3: 1086-1089.

13. Robins, D. M., R. Axel and A. S. Henderson (1981) Chromosome structure and DNA sequence alterations associated with mutation of transformed genes. J. Mol. Appl. Genet. 1: 191-203.

14. Jackson, J., T. Lowy, M. Ostrander, A. Pellicer, J. Roberts, D. Robins, G.-K. Sim, R. Sweet, B. Wold, S. Silverstein and R. Axel (1980) Stable and unstable expression of genes in DNA transformed cells. pp. 181-199. In Mobilization and Reassembly of Genetic Information, Miami Winter Symposia - Vol. 17 (W. A. Scott, R. Werner, D. R. Joseph and J. Schultz, eds.), Academic Press, NY.

15. Ostrander, M., S. Vogel and S. Silverstein. 1982. Phenotypic switching in cells transformed with the Herpes simplex virus thymidine kinase gene. Mol. Cell. Biol. 2: 708-714.

16. Sweet, R., J. Jackson, T. Lowy, M. Ostrander, A. Pellicer, J. Roberts, D. Robins, G.-K. Sim, B. Wold, R. Axel and S. Silverstein. 1981. The expression, arrangement and rearrangement of genes in DNA transformed cells. pp 205-219. In Genes, Chromosomes and Neoplasia, F. E. Arrighi, P. N. Rao and E. Stubblefield (eds.), Raven Press, NY.

17. Thacker, J., P. G. Debenham, A. Stretch and M. B. T. Webb (1983)

The use of a cloned bacterial gene to study mutation in mammalian cells. Mutat. Res. 11: 9-23.

18. Tindall, K. R. and A. W. Hsie (1983) Detection of deletion mutations at the gpt locus in pSV2gpt transformed CHO cells. In Cellular Responses to DNA Damage, UCLA Symposia on Molecular and Cellular Biology - new series, Vol. 11 (E. C. Friedberg and B. R. Bridges, eds.), Alan R. Liss, Inc., NY (in press).

19. Mulligan, R. C. and P. Berg (1980) Expression of a bacterial gene in mammalian cells. Science 209: 1422-1427.

20. Hsie, A. W., P. A. Brimer, T. J. Mitchell and D. G. Gosslee (1975) The dose-response relationship for ethyl methanesulfonate induced mutations at the hypoxanthine-guanine phosphoribosyl transferase locus in Chinese hamster ovary cells. Somat. Cell Genet. 1: 247-261.

21. O'Neill, J. P., P. A. Brimer, R. Machanoff, G. P. Hirsch and A. W. Hsie (1977) A quantitative assay of mutation induction at the hypoxanthine-guanine phosphoribosyl transferase locus in Chinese hamster ovary cells (CHO/HGPRT system): Development and definition of the system. Mutat. Res. 45: 91-101.

22. Calos, M. P., J. S. Lebrowski and M. R. Botchan. 1983. High mutation frequency in DNA transfected into mammalian cells. Proc. Natl. Acad. Sci. U.S.A. 80: 3015-3019.

TABLE 1

UV- and EMS-Induced Mutation and Relative Cytotoxicity in the AS52 (*gpt*$^+$) and CHO-K1-BH4 (*hgprt*$^+$) Cell Lines

	Mutation Frequency (TG^r mutants/10^6 clonable cells)		Cytotoxicity (% Relative Survival)	
	AS52	CHO-K1-BH4	AS52	CHO-K1-BH4
UV Dose (J/m^2)[a]				
0	23	4	100	100
1.5	169	112	79.0	104
2.5	128	89	70.6	94.2
3.0	316	263	67.2	67.9
4.5	423	402	53.1	67.9
5.0	367	214	40.1	57.8
6.0	508	408	48.5	41.9
7.5	614	338	16.2	25.3
9.0	640	587	11.6	10.8
[EMS] (ug/ml)[b]				
0	31	12	100	100
75	85	130	87.8	86.8
150	148	238	77.4	83.4
300	246	360	60.2	48.2
600	523	739	37.6	23.5

a) pooled results from 2 independent experiments

b) average results from 2 independent experiments

TABLE 2

UV- and EMS-Induced TG^r Mutant Frequency in the AS52 (*gpt*$^+$) and CHO-K1-BH4 (*hgprt*$^+$) Cell Lines

	TG^r Mutants/10^6 Clonable Cells	
	per J/m^2 UV-light	per ug/ml EMS
AS52	74	0.82
CHO-K1-BH4	54	1.2
Ratio $XGPRT^-$ to $HGPRT^-$	1.4	0.70

TABLE 3

Results of Southern Blot Hybridization Analysis of 30 TG^r Clones Derived from the AS52 Cell Line

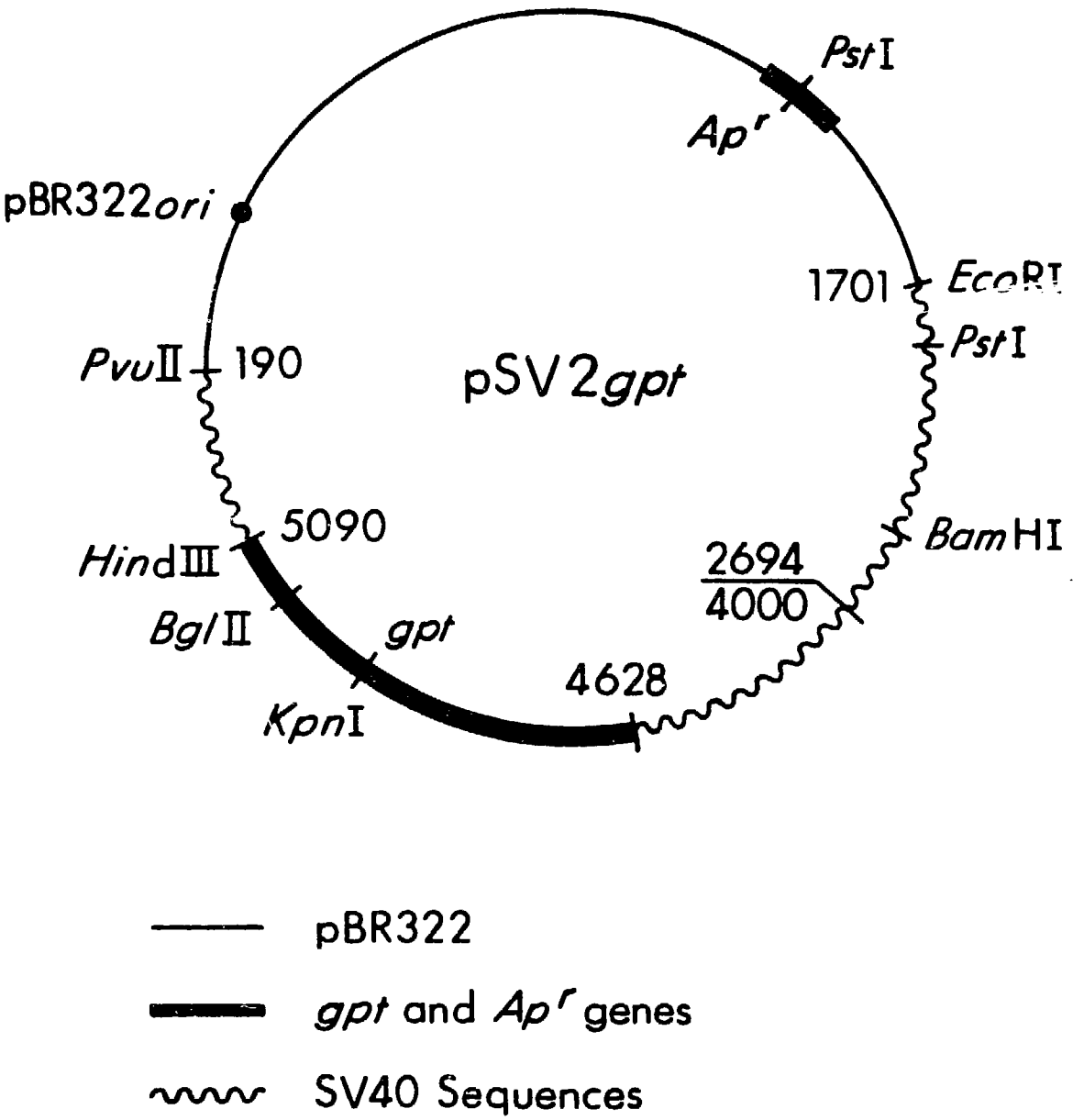
Type of Mutant (no. analyzed)	No detectable alteration	Type of Mutation		
		1-4 KB deletion	Total plasmid deletion	Detectable minor alteration
Spontaneous (19)	5	9	4	1
UV-light induced (5)	5	-	-	-
EMS-induced (6)	5	-	1	-

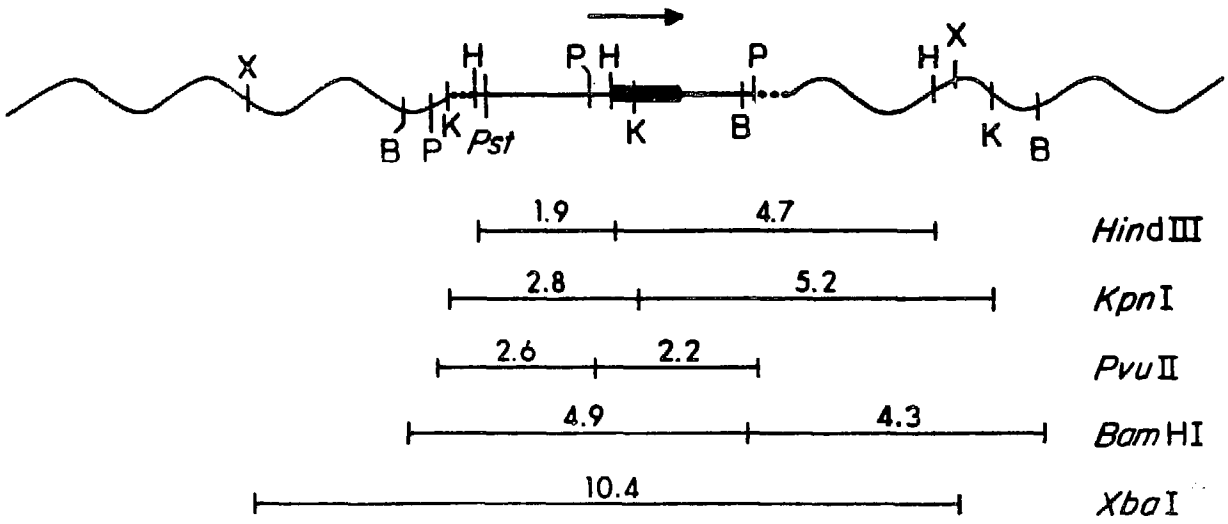
FIGURE LEGENDS

Figure 1. Organization of the pSV2gpt vector. The pBR322 sequences in this vector extend from the PvuII to the EcoR1 sites and include the plasmid origin of replication and the ampicillin resistance gene. SV40 sequences are numbered relative to the SV40 origin of replication. The E. coli gpt gene is positioned for expression in mammalian cells using the SV40 early promoter. Redrawn from (16).

Figure 2. Map of the integrated pSV2gpt sequences in the AS52 line. Reprinted from (20).

Figure 3. Results of Southern blot analysis of 19 spontaneous TG^r AS52 mutants. The upper line is the map of the integrated pSV2gpt sequences in AS52 cells. Solid bars indicate the size of any deletions observed. Dotted lines indicate the possible lateral boundaries of the deletions.


pSV2gpt
PstI
Ap^r
pBR322ori
1701
EcoRI
PstI
PvuII
190
BamHI
5090
2694
4000
HindIII
BglII
gpt
4628
KpnI
pBR322
gpt and Ap^r genes
SV40 Sequences



Genomic DNA
pSV2 sequences
gpt gene
Direction of transcription
1kb

FIG 2

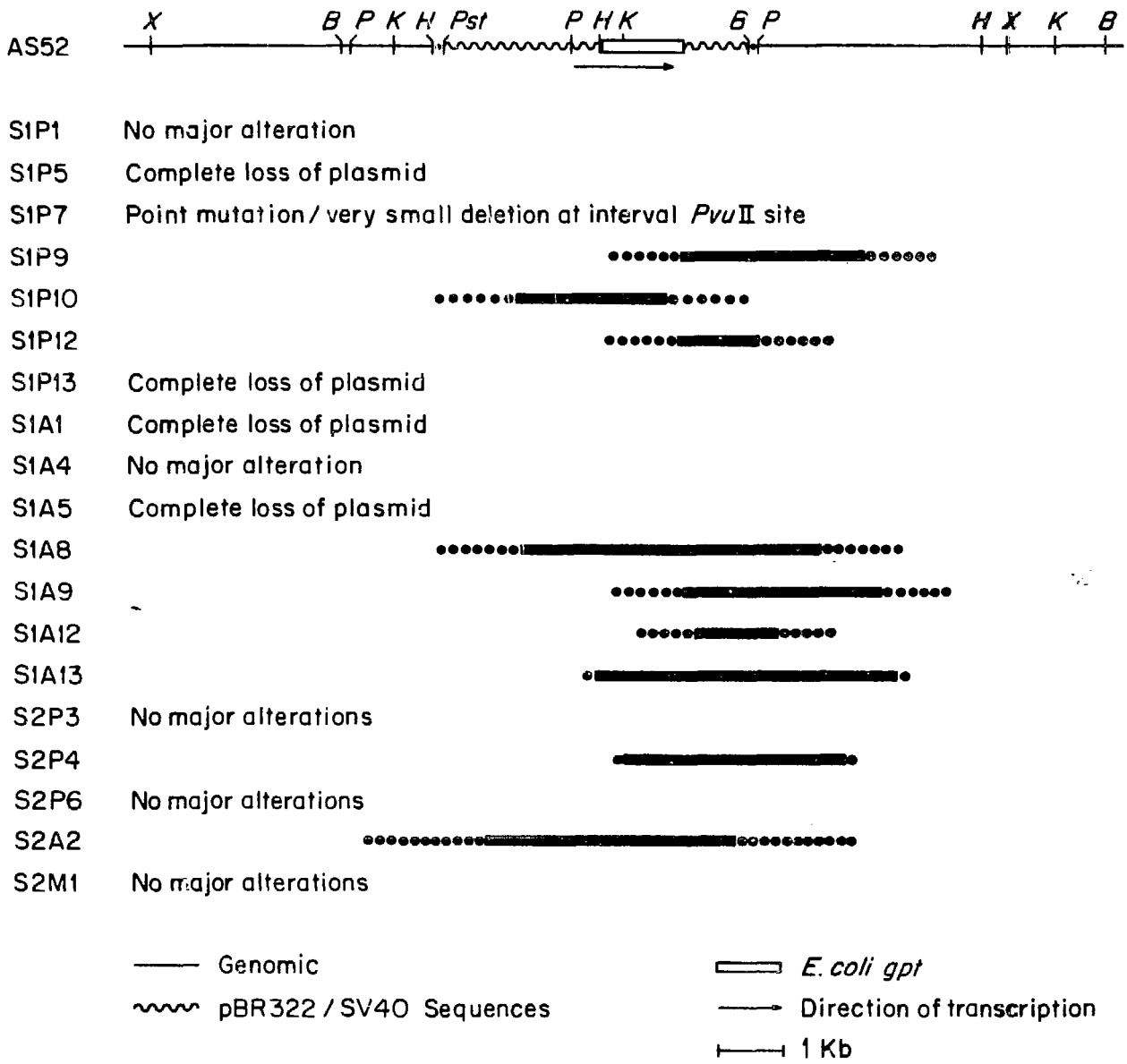

X
B P K H Pst
P H K
B P
H X K B
AS52
S1P1 No major alteration
S1P5 Complete loss of plasmid
S1P7 Point mutation / very small deletion at interval PvuII site
S1P9
S1P10
S1P12
S1P13 Complete loss of plasmid
S1A1 Complete loss of plasmid
S1A4 No major alteration
S1A5 Complete loss of plasmid
S1A8
S1A9
S1A12
S1A13
S2P3 No major alterations
S2P4
S2P6 No major alterations
S2A2
S2M1 No major alterations
Genomic
pBR322 / SV40 Sequences
E. coli gpt
Direction of transcription
1 Kb

FIG 3