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Variable Stability of a Selectable Provirus after Retroviral Vector Gene Transfer into Human Cells

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Human lymphoblasts deficient in the enzyme hypoxanthine-guanine phosphoribosyltransferase (HPRT) were infected with an amphotropic helper-free retroviral vector expressing human HPRT cDNA. The stability and expression of the HPRT provirus in five cell lines with different proviral integration sites were examined by determining HPRT mutation and reversion frequencies and by blot hybridization studies. Mutation to the HRPT-negative phenotype occurred at frequencies of approximately 4×10^{-5} to 3×10^{-6} per generation. Most mutations in each of the five cell lines were associated with partial or complete deletions or rearrangements of the provirus. Several mutants retained a grossly intact HPRT provirus, and in one such mutant HPRT shutdown resulted from a revertable epigenetic mechanism that was not associated with global changes in proviral methylation. Therefore, mutation and shutdown of the HPRT provirus in human lymphoblasts result from mechanisms similar to those reported for several other avian and mammalian replication-competent retroviruses.

Defective forms of eucaryotic retroviruses have recently become useful for studying the transfer of genetic information into mammalian cells because of their very high efficiency of infection, their large capacity for added sequences, and their fairly well-understood mechanisms of infection, integration, and gene expression in recipient cell genomes (15, 16, 21, 24, 28, 32, 34, 35, 43, 44). However, it is known that retroviral infection of eucaryotic cells can be accompanied by proviral instability, as shown by the occasionally high rates of reversion and shutdown of gene expression and by changes in proviral structure, including deletions, point mutations, losses of entire proviruses, and changes in proviral methylation (1, 3, 6, 7, 9–12, 17, 19, 22, 23, 25, 26, 30, 31, 39–42, 47). It has not been clear how many of these instabilities are due to error-prone replication and how many are due to nonreplicative mechanisms. It is known that rat cells expressing an avian sarcoma virus revert to the nontransformed phenotype by several mechanisms, including loss of the entire provirus and nonconditional mutations in the *src* transforming gene (41). However, similar direct studies of the stability of nontransforming proviruses have not been extensive due to the paucity of easily selectable proviral gene products.

We investigated the stability of proviral organization and expression by using nonproducer, hypoxanthine-guanine phosphoribosyltransferase (HPRT)-deficient human lymphoblast cell lines genetically transformed to the $HPRT^+$ phenotype by infection with an HPRT retroviral vector (21). In this study we took advantage of the facility and efficiency of selection both for and against cells expressing HPRT activity in tissue culture (13, 46), thereby allowing the detection and characterization of rare genetic events that affect the expression of the HPRT gene. Evidence from Southern blots, mutation and reversion frequencies, and enzyme activities indicated that in human HPRT-deficient lymphoblasts infected with an HPRT retroviral vector, integrated HPRT proviruses are relatively stable, showing frequencies of mutation from $HPRT^+$ to $HPRT^-$ ranging from 3×10^{-6} to 4×10^{-5} per generation. The mechanisms of mutation to the $HPRT^-$ phenotype vary in cell lines carrying proviruses integrated at different sites and include a variety of deletions, rearrangements, and epigenetic events. These findings may be of use in developing an understanding of the fate and expression of defective retroviral vectors in human and other mammalian cells.

MATERIALS AND METHODS

Cells and culture. Human Lesch-Nyhan HPRT-deficient lymphoblasts (46) transformed by Epstein-Barr virus were grown in RPMI 1640 medium containing 10% fetal calf serum at 37°C in 5% CO_2. The enzyme-deficient cells were infected with a transmissible HPRT vector and were selected and characterized as described previously (46). Independently isolated $HPRT^+$ cell lines 1, 9, 11, 13, and 16 were chosen for further characterization and were designated the parent cell lines in this study.

Selection of $HPRT^-$ cells was performed with 20 μM 6-thioguanine, and the selection medium for $HPRT^+$ cells contained 10^{-4} M hypoxanthine, 2.5×10^{-7} M aminopterin, and 5×10^{-4} M thymidine (HAT) added to the medium described above. Mutation frequencies were measured by growing cells in HAT medium, withdrawing them from HAT medium for periods of 1 to 100 days, and plating them in 0.3% soft agar containing 2 μM thioguanine. The number of resistant colonies, corrected for plating efficiency, was determined after 10 to 14 days, and the mutation frequency per generation was calculated from the initial slope of the curve correlating the time without selection with the number of mutants. Reversion from $HPRT^-$ to $HPRT^+$ was measured by analogous procedures, using HAT selective media.

Parental cell lines were determined to be nonproducers (i.e., not infected with a competent retrovirus) by growing the infected $HPRT^+$ cells to a density of 0.5×10^6 cells per ml, replacing the medium with fresh medium containing 4 μg of Polybrene per ml, harvesting the medium 16 h later, and testing for the presence of HPRT infectious units on a

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TABLE 1. HPRT levels and mutation and reversion frequencies of HPRT-infected cell lines

Cell line	HPRT level (% of wild type)	Mutation to thioguanine resistance		HAT^r reversion		Second-cycle mutation to thioguanine resistance	
		Frequency	HPRT level (% of wild type)[a]	Frequency	HPRT level (% of wild type)	Frequency	HPRT level (% of wild type)[a]
1	20	3×10^{-6}	<0.5[b]	$<10^{-7}$			
9	11	3×10^{-5}	<0.5[c]	$<10^{-7}$			
11	18	3×10^{-6}	<0.5[d]	$<10^{-7}$			
13	23	4×10^{-5}	<0.5[d]	$<10^{-7}$			
16	5	1.5×10^{-5}	<0.5[d]	1.3×10^{-4}	4[d]	3.3×10^{-3}[e]	<0.5

[a] The levels were indistinguishable from the level in the original Lesch-Nyhan parent cell line.
[b] Determined by using two clones; HPRT activities were measured individually.
[c] Determined by using six clones; HPRT activities were measured individually.
[d] Determined by using five clones; HPRT activities were measured individually.
[e] Determined by using cell line 16b revertant 3.

recipient lawn of HPRT-deficient human SV40-transformed Lesch-Nyhan (LNSV) cells or rat 208F cells. Two cell lines (cell lines 16 and 11) were infected with competent 1504A virus (21) and grown for 2 weeks before the supernatants were assayed for HPRT virus production.

For mutation and reversion studies, colonies were plated after no more than 1 day out of HAT or thioguanine selective conditions in order to minimize the possibility of picking sibling clones.

HPRT activities. Cells were grown to mid- to late-log phase and harvested, and cell extracts were prepared and assayed for HPRT activity as described previously (14, 46), using [^{14}C]-hypoxanthine as a substrate. HPRT activities were normalized to levels of adenosine phosphoribosyl transferase (46).

DNA and RNA preparation, restriction enzyme digests, and Southern blots. DNA and RNA were prepared as described previously (21). Restriction enzymes *Sst*I, *Sac*I, *Pvu*II, *Eco*RI, *Bam*HI, *Hpa*II, and *Msp*I were purchased from Amersham Corp., New England BioLabs, Inc., or Bethesda Research Laboratories and were used as recommended by the manufacturers. Agarose gel analysis of enzyme-digested DNA and transfer of DNA to nitrocellulose filters by Southern blotting (33) were performed as described previously (14, 46). Hybridization with a nick-translated probe (18) was performed in 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate, pH 7) at 68°C. The filters were washed initially with 2× SSC and finally with 0.2× SSC at 68°C. Hybridization probes were purified free of plasmid vector sequences by agarose gel electrophoresis after restriction enzyme digestion and nick translated to a specific activity of approximately 2×10^8 cpm/μg. The following DNA probes were used: for HPRT, the *Pst*I-to-*Rsa*I fragment from cDNA plasmid p4aA8 containing the full coding portion of the cDNA and a portion of the untranslated 3′ region (bases 1 to 890) (14); for the long terminal repeat (LTR), the double LTR fragment cloned with R1 linkers in pLTR10 (46).

RESULTS

Five previously described (46) human, Epstein-Barr virus-transformed, nonproducer lymphoblast cell lines derived from a Lesch-Nyhan patient and made HAT resistant by infection with a transmissible HPRT retroviral vector were tested for their frequency of mutation to thioguanine resistance (i.e., HPRT$^-$). These cell lines had HPRT activity ranging from 5 to 23% of the wild type levels, and all contained one detectable integrated copy of the HPRT provirus (46). Their properties, mutation frequencies, and HPRT enzyme levels are shown in Table 1. Thioguanine was used as a selective agent because it is known to give few, if any, HPRT$^+$ thioguanine-resistant cells, in contrast to azaguanine (27, 29). Table 1 also shows the frequencies with which the thioguanine-resistant (HPRT$^-$) mutants reverted to the HAT-resistant HPRT$^+$ phenotype.

The mutation rate of control cell line WI-L2 during selection of the HPRT-negative phenotype with thioguanine has been reported to be anywhere from less that 2×10^{-7} to 10^{-6} per cell generation (4, 5, 36–38; Willis, unpublished data), values that depend on the history of the cell line, the cell density, the concentration of the selective agent, and other culture parameters. Using the conditions described here, workers in our laboratories have found that the mutation frequency of cell line WI-L2 is approximately 10^{-6} per cell generation. The mutation frequencies for the five parent HPRT-positive Lesch-Nyhan lymphoblast transformants ranged from 4×10^{-5} to 3×10^{-6} per generation, values that are approximately equal to, but probably somewhat higher than, the mutation frequency reported for the HPRT locus in WI-L2 cells and for a variety of other single-copy genes in tissue culture cells.

In order to determine the mechanism of the mutation in these cells, we examined the structure of the HPRT provirus by Southern blotting DNAs from the thioguanine-resistant cells. The organization of the HPRT provirus in HPRT-deficient cells infected by the transmissible HPRT vector is shown in Fig. 1. Digestion of DNAs from the HPRT-infected parent cells with *Sac*I, its isoschizomer (*Sst*I), or other enzymes that cut an intact, unrearranged HPRT provirus only in the LTRs would be expected to reveal a single 4-kilobase (kb) band with an HPRT probe, while digestion with *Bam*HI should reveal one band of variable size (but always larger than 2.6 kb by an amount dependent on the site of proviral integration and the location of the nearest *Bam*HI site in the flanking host sequences). Similarily, digestion of cellular DNA with *Eco*RI and hybridization with an HPRT probe should reveal one band of variable size, always larger than 4.6 kb, since there are no *Eco*RI sites within the provirus. All of these patterns, of course, are superimposed on fragments derived from the endogenous, nonfunctional HPRT gene sequences known to be present in these cells (46).

With Moloney murine sarcoma virus LTR probes that did not hybridize to a significant extent with human DNA under our standard conditions of stringency, *Sst*I-digested DNA should have revealed the 4-kb band identical to the band visualized with the HPRT probe, in addition to another major band of variable size. Hybridization with the same LTR probes to blots of *Bam*HI-digested DNA should have

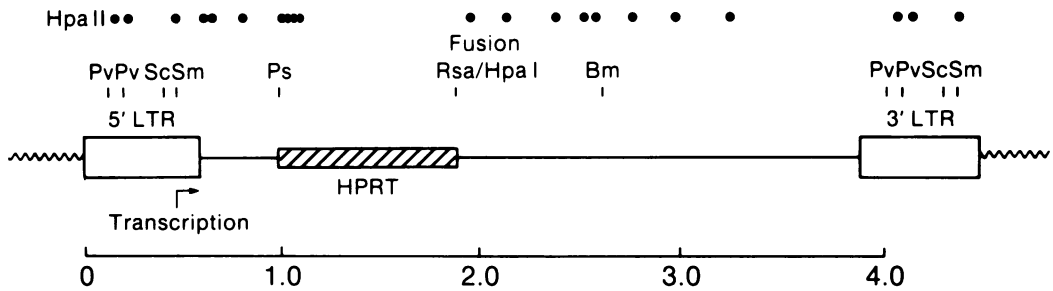

FIG. 1. Map of the HPRT provirus in infected cells. The open boxes represent the LTR sequences, and the cross-hatched box represents the HPRT sequence. The *Hpa*II (and hence *Msp*I) restriction enzyme sites are indicated. The other sites include *Pvu*II (Pv), *Sac*I or *Sst*I (Sc), *Sma*I (Sm), *Pst*I (Ps), and *Bam*HI (Bm) sites. The scale is in kilobase pairs. The cellular DNA sequences immediately adjacent to the 5′ and 3′ LTR sequences are indicated by wavy lines.

revealed two fragments of variable size, one of which corresponded to the same fragment detected with an HPRT probe and one of which was unique. Blots of cellular DNA digested with *Eco*RI hybridized to the LTR probe should have revealed one band identical to the band revealed by the HPRT probe and larger than 4.6 kb.

Combinations of restriction enzymes and hybridization probes were used to characterize the structure of the HPRT provirus in the five parent cell lines grown with and without continued HAT selection in mutants and revertants.

Cell line 13. In six HPRT-negative mutants derived from tetraploid (46) parent cell line 13 (three mutants derived from each of two separate experiments), all of the HPRT and LTR proviral sequences were lost, and in the one mutant line which we examined by karyotype analysis the proviral loss was accompanied by the loss of chromosome 4 (data not shown). No proviral rearrangements were detected in parent cells after prolonged growth for 22 generations without selection.

Cell line 9. The results obtained with tetraploid cell line 9 are shown in Fig. 2. Mutants 9a, 9b, and 9c and mutants 9d, 9e, and 9f were derived from two separate experiments. As was the case with cell line 13, there was no detectable difference between the DNAs from cells cultured with HAT selection and the DNAs from cells cultured without HAT selection for 22 generations (Fig. 2, lanes 2 and 3). All six HPRT$^-$ mutants (mutants 9a, 9b, 9c, 9d, 9e, and 9f) (Fig. 2, lanes 4 through 9) had lost most or all of a 7.5-kb *Bam*HI fragment containing the HPRT sequences found in the parent cell lines (lanes 2 and 3), leaving only the endogenous HPRT gene pattern (lane 1).

The same filter containing *Bam*HI-digested DNA hybridization to the LTR probe showed that two of the six revertants had also lost the LTR sequences (Fig. 2, lanes 13 and 16). Three of the mutants (mutants 9a, 9d, and 9f) (lanes 12, 15, and 17) produced LTR patterns which were different from those of the parent cells but very similar to each other, retaining the parental HPRT-negative, LTR-positive, 13-kb *Bam*HI fragment and also showing two new *Bam*HI fragments (approximately 15 and 5 kb). Mutant 9c (lane 6) retained only the 15-kb LTR-positive fragment (lane 14). Since the same filter was used for hybridization to both the

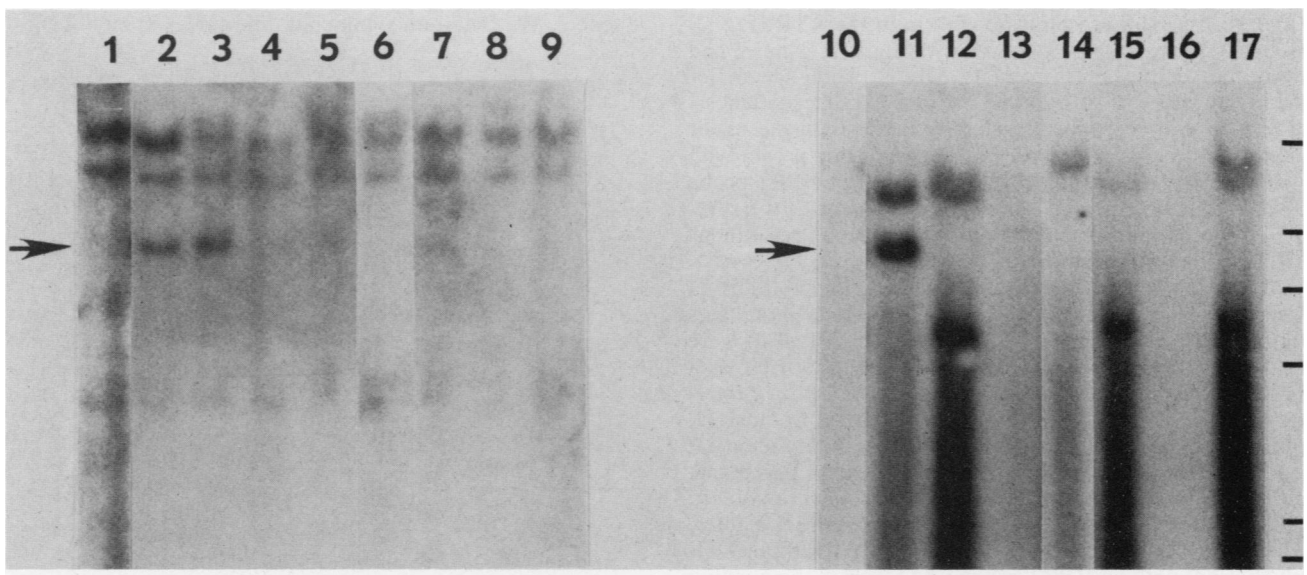

FIG. 2. Hybridization of HPRT (lanes 1 through 9) and LTR (lanes 10 through 17) probes to blots of *Bam*HI digests of DNAs from cell line 9 and its derivatives. Lanes 1 and 10, Cell line 1547, the original HPRT$^-$ cell line which was infected with HPRT virus to yield cell line 9; lane 2, cell line 9 maintained for 22 weeks without HAT selection; lanes 3 and 11, cell line 9 maintained with HAT selection; lanes 4 through 9 and 12 through 17, HPRT$^-$ mutants 9a, 9b, 9c, 9d, 9e, and 9f, respectively. The arrows indicate the approximately 7.5-kb band in cell line 9 (lanes 2, 3, and 11) which was apparent in the HPRT and LTR hybridizations but was not detectable in the HPRT$^-$ mutants (lanes 4 through 9 and 12 through 17). The markers indicated on the right are λ *Hin*dIII fragments (23.6, 9.6, 6.6, 4.3, 2.3, and 2.0 kb from top to bottom).

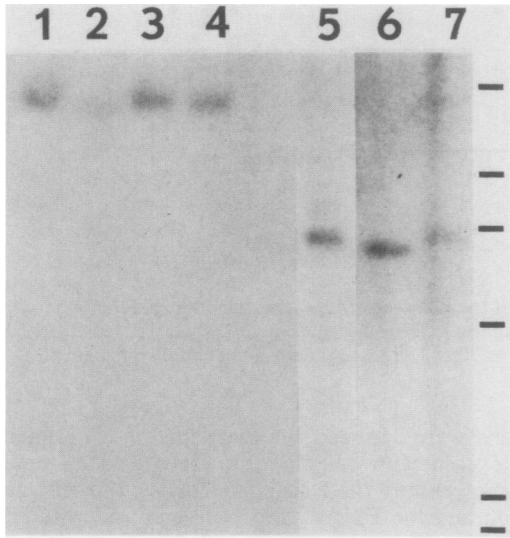

FIG. 3. Hybridization of the LTR probe to blots of *Bam*HI digests (lanes 1 through 4) and *Eco*RI digests (lanes 5 through 7) of DNAs from cell line 1 and its derivatives. Lanes 1 and 5, Parent cell line 1 maintained with HAT selection; lanes 2 and 6, HPRT$^-$ mutant cell line 1e; lanes 3 and 7, HPRT$^-$ mutant cell line 1f; lane 4, parent cell line 1 maintained without HAT selection for 22 weeks. The markers indicated on the right are λ *Hin*dIII fragments (see the legend to Fig. 2).

LTR and the HPRT probes, the low-molecular-weight smear found in several mutants, as well as in the parent cell lines, cannot be explained by sample degradation or variations in sample loading.

Cell line 1. The results obtained with diploid cell line 1 are shown in Fig. 3. There was no detectable difference in proviral structure between the parent cell line maintained in HAT medium and the cell line grown for 22 generations without selection for the HPRT$^+$ phenotype (Fig. 3, lanes 1 and 4). We characterized two HPRT-negative clones, clones 1e and 1f, by Southern blotting with both the LTR (Fig. 3) and HPRT probes (data not shown). Mutant 1f (lanes 3 and 7) was indistinguishable from its parent, while the major band in mutant 1e in both *Eco*RI and *Bam*HI digests (lanes 2 and 6) migrated reproducibly slightly faster than the major band of the other revertant or of the parent line grown with or without HAT selection. Hybridization of the HPRT probe to *Eco*RI digests showed that HPRT sequences were retained in both mutants and migrated in the same position as the LTR-hybridizing band.

Cell line 11. The results obtained with diploid cell line 11 are shown in Fig. 4. Mutants 11a, 11b, and 11c (Fig. 4, lanes 2 through 4) and mutants 11e and 11f (lanes 5 and 6) were derived from two separate experiments. Southern blots of cellular DNAs from all five mutants digested with *Bam*HI (Fig. 4A) or with *Sst*I (Fig. 4B) and hybridized to the LTR probe showed patterns that were identical or nearly identical to each other but different from the pattern for the parent line (lane 1). All of the mutants were completely devoid of proviral HPRT sequences (data not shown). In *Sst*I digests, a fragment (Fig. 4B, arrow) which hybridized with an LTR probe but not with an HPRT probe was found in the position of one of the LTR bands in the parental cell line. We obtained similar results with Southern blots of cellular DNA digested with *Pvu*II, which also cut the HPRT proviral DNA only in the LTRs (Fig. 1) (data not shown). Such patterns are most easily explained by homologous recombination between the 5′ and 3′ LTRs, resulting in the excision of the single proviral *Sst*I or *Pvu*II fragment and the persistence of unaltered LTR fragments.

Cell line 16. Figure 5 shows the mutation and reversion frequencies for diploid line 16. Mutants 16a, 16b, and 16c (Fig. 5, lanes 3 through 5) and mutants 16e and 16f (lanes 6 and 7) were derived from two separate experiments. The same *Bam*HI digests were hybridized to the LTR (Fig. 5a) and HPRT (Fig. 5b) probes. The HPRT and LTR blots showed that the parent cell line (lanes 1 and 2) had an LTR-containing proviral band at 4.3 kb (Fig. 5a, arrow B) that also hybridized to the HPRT probe (Fig. 5b, arrow C). As expected (Fig. 1), the parent cell line also had a second LTR-hybridizing band, at approximately 11 kb. This arrangement was not detectably altered in two HPRT$^-$ mutants (lanes 4 and 5). However, in mutant 16a (lane 3), the upper LTR band was apparently increased in size, and in cell lines 16e and 16f (lanes 6 and 7) the HPRT-containing 4.3-kb band was completely lost and the upper band was reduced in size in at least one of these cell lines (lane 7).

HPRT$^-$ mutant 16b (lane 4) reverted to HAT resistance (Table 1), and the results of hybridization to digests of DNAs from several such HPRT$^+$ revertant lines (cell lines 16br1, 16br2, and 16br3) are shown in Fig. 5, lanes 8 through 10. In addition, the results obtained with DNA samples from four mutant cell lines derived from a second cycle of thioguanine selection of cell line 16br3 (lane 10) are shown in lanes 11 through 14. The HPRT and LTR blots showed no detectable rearrangement of the HPRT proviral sequences in these experiments after several sequential cycles between the HPRT-positive and -negative phenotypes.

Because of the stable Southern blot pattern and the very high frequency of HPRT phenotype switching, it seems likely that the modulation of HPRT gene expression in mutant 16b, its revertants, and second-cycle mutants is regulated by epigenetic events. Therefore, we examined the methylation pattern of the HPRT provirus after digestion with *Bam*HI and either *Hpa*II or *Msp*I (2) (see Fig. 1 for the locations of restriction enzyme sites in the proviral sequence). We found no significant differences in any of these

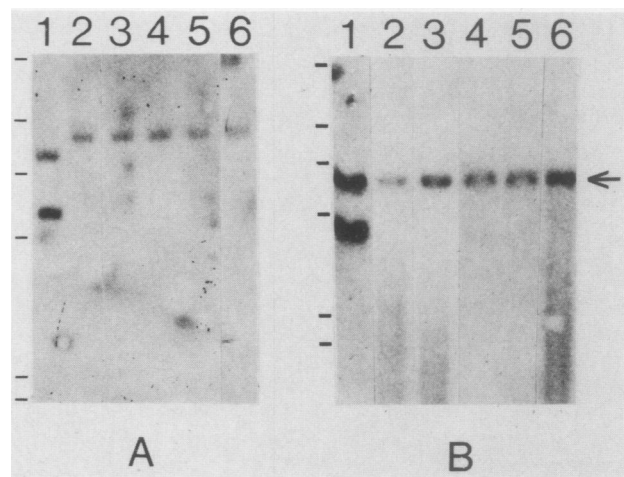

FIG. 4. Hybridization of the LTR probe to blots of digests of DNAs from cell line 11 and its derivatives. Lanes 1, Parent cell line 11; lanes 2 through 6, HPRT-negative mutant lines 11a, 11b, 11c, 11e, and 11f, respectively. (A) *Bam*HI digests. (B) *Sst*I digests. The arrow indicates the band that was conserved in the parent and mutant lines in the *Sst*I digests. The markers used were λ *Hin*dIII fragments (see the legend to Fig. 2).

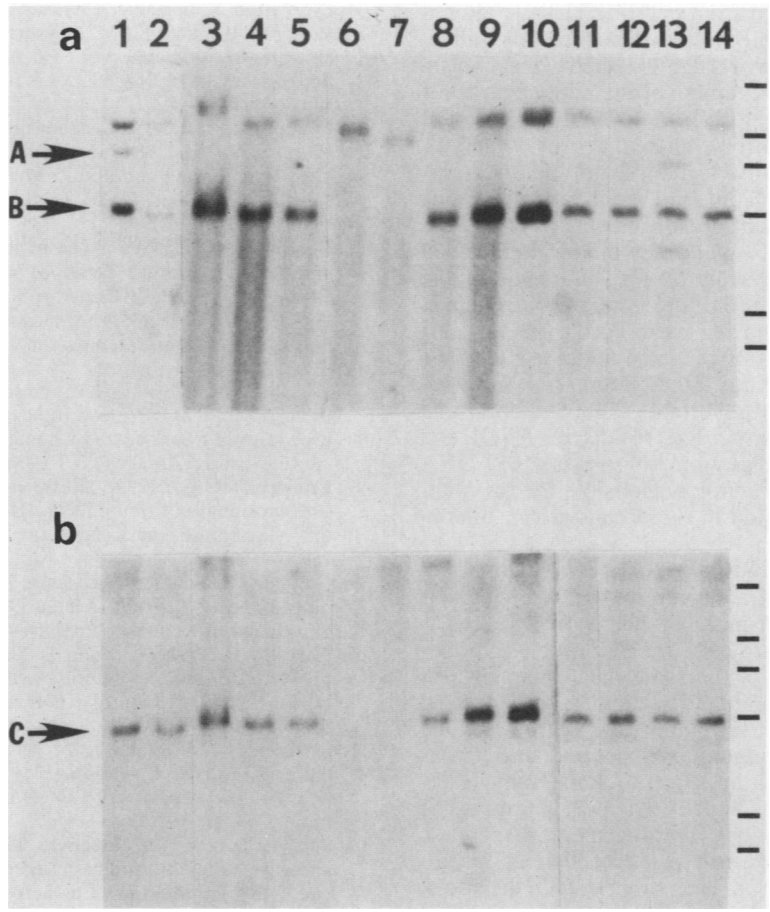

FIG. 5. Hybridization of LTR (a) and HPRT (b) probes to *Bam*HI digests of DNAs from cell line 16 and its derivatives. Lane 1, Cell line 16 preparation 2, which was made after extended growth with HAT selection (approximately 100 generations after infection of the parent HPRT$^-$ cell line with the HPRT virus); lane 2, initial DNA preparation of cell line 16 (approximately 25 generations after vector infection); lanes 3 through 7, HPRT$^-$ mutants 16a, 16b, 16c, 16e, and 16f, respectively; lanes 8 through 10 HPRT$^+$ revertants of mutant 16b (revertants 16br1, 16br2, and 16br3, respectively); lanes 11 through 14, HPRT$^-$ thioguanine mutants of revertant 16br3 (mutants 16br3rr1, 16br3rr2, 16br3rr3, and 16br3rr4, respectively). Arrow A indicates the extra LTR-hybridizing band at around 8 kb observed in parent cell line 16 after extended growth with HAT selection. The other arrows indicate the indistinguishable proviral bands of about 4 kb, which hybridized to the LTR (arrow B) and HPRT (arrow C) probes and were maintained in most of the cell lines. The markers used were λ *Hin*dIII fragments (see the legend to Fig. 2).

cell lines (data not shown), suggesting that the switch in these cells from HPRT gene expression to shutdown is not obviously correlated with global changes in the extent of methylation of the HPRT proviral sequences.

DISCUSSION

The HPRT gene in the form of transmissible retroviral vectors offers some major advantages as a model gene for the study of the stability of proviruses in mammalian cells. It provides an easily selectable, nontransforming provirus-derived gene function whose stability through repeated cycles of mutation and reversion of nonproducer cells permits distinction between replication-derived instability and proviral instability. Information obtained from such a selectable system ought to be relevant to other genes introduced into mammalian cells by similarly designed retroviral vectors, as well as to the parent retroviruses themselves.

The HPRT provirus which we used is relatively stable, showing frequencies of mutation to the HPRT$^-$ phenotype of approximately 4×10^{-5} to 3×10^{-6} per cell generation, a value slightly to moderately higher than the value obtained from the HPRT locus in the control WI-L2 cells. Nevertheless, the HPRT provirus is susceptible to several kinds of rearrangements or epigenetic events that accompany shutdown of proviral HPRT expression. In one of the parent cell lines (cell line 13) which was tetraploid before selection of HPRT-negative mutants, proviral loss was associated with loss of chromosome 4. We did not distinguish between the possibility that the provirus was eliminated through chromosome loss and the possibility that it was eliminated through coincidental but unrelated proviral excision. Similarly, whether chromosome loss is responsible for proviral loss in some of the mutants of the other tetraploid parent cell line (cell line 9) is not clear, but the persistence and modification of LTR sequences, together with the complete loss of HPRT sequences in several of the other mutants derived from cell line 9, indicate that proviral deletions and rearrangements occur in at least some mutants in this cell line. Less extensive changes seem to have occurred in the two mutants of parent cell line 1; one apparently resulted in a small detectable deletion, and the other resulted in a grossly intact provirus.

All of the mutants derived from cell line 11 have altered structures that are compatible with homologous recombination events between the proviral LTRs. Such a mechanism has been suggested for the reversion of the dilute coat color mutation in DBA/2J mice which results from the excision of an integrated mutagenic ecotropic leukemia virus (7) and for the reversion from nontransformed phenotype to transformed phenotype of a cell line in which an exogenous provirus interrupted a resident transforming gene (40). Similarly, some of the mutants of parent line 16 retained a grossly unaltered provirus, while others, like some of the mutants of cell line 9, lost the HPRT sequence but retained LTR sequences, albeit at times in rearranged form. At least one of the mutants (mutant 16b) obviously retained all of the sequences required for proviral HPRT expression since we were able to select $HPRT^+$ revertants (16br mutants) and second-cycle $HPRT^-$ mutants. The results of *Hpa*II and *Msp*I restriction enzyme digestion do suggest that DNA methylation is not a major factor responsible for the shutdown of viral gene expression in these cells (Jolly, unpublished data).

Our data indicate that a murine retrovirus-based amphotropic HPRT provirus integrated into human lymphoblasts after infection with a transmissible helper-free vector is relatively stable, but that rare cells are susceptible to proviral shutdown by a number of the same mechanisms which are known to modify avian and murine proviral structure and expression in other cells. Since only few of the isolated mutants contained grossly intact proviruses and since it was not possible (except for cell line 16b) to isolate any HAT-resistant reversions, we concluded that most mutations in this system involve deletions and rearrangements rather than single-base mutations. Since the parent cell lines in this study presumably differed from each other only in the site of proviral integration, we inferred that the various modes of proviral instability are influenced by the site of integration of the provirus or by flanking cellular sequences. Such a positional effect is not surprising in view of the well-known effect of integration site on some proviral expression (8, 11, 12).

Studies of the stability of integrated proviruses not only may be helpful in characterizing the general mechanisms of recombination involved in viral integration, but also are important components of studies to test the feasibility of retrovirus-mediated gene therapy models (20, 45). A reasonable requirement for such an approach to therapy is that the structure and expression of the genetic modification be stable. Our results indicate that the HPRT provirus is indeed reasonably stable in diploid human lymphoblasts, but that the several genetic and epigenetic mechanisms known to modify other proviruses also cause HPRT shutdown at detectable rates.

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