Insertion Mutagenesis of Embryonal Carcinoma Cells by Retroviruses

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Abstract. *Mutagenesis was studied in cultured F9 embryonal carcinoma cells infected with a variant of Moloney murine leukemia virus. Proviral insertion induced the inactivation of the hypoxanthine phosphoribosyltransferase locus, and the virus was used to isolate the mutated genes rapidly. Mutagenesis by these methods may be useful for the genetic dissection of the various mammalian cell phenotypes.*

Embryonal carcinoma (EC) cells, the pluripotent stem cells of teratocarcinomas, have been used to study certain cellular and molecular aspects of early mammalian development (*1–4*). These cells share many properties with early embryonic cells, such as the ability to differentiate in vivo and in vitro into many cell types (*3, 4*) and to participate in the development of chimeric animals (*5–7*). Furthermore, EC cells are available as clonal cell lines and therefore can be readily subjected to various genetic manipulations such as mutagenesis, gene transfer, and cell fusion. The conversion of EC cells into differentiated cells could be studied by isolating mutant EC cells that behave abnormally with regard to differentiation and then isolating the genes responsible for the mutant phenotypes. Although it is now possible to select for chemically induced or spontaneous mutants of EC cells that have lost the ability to differentiate in response to certain inducers (*8–12*), it is not simple to isolate the mutant genes of interest. We have therefore investigated the possibility of using retroviruses as insertion mutagens in cultured EC cells.

In prokaryotes, insertion mutagenesis by transposable elements has been extremely useful in the definition and isolation of genes of various biological function (*13*). In yeast, maize, and *Drosophila* genes, many mutations have been shown to result from the insertion of transposable elements (*14*). In mammalian cells, retroviruses can act as insertion mutagens since an obligate part of their life cycle involves the integration of proviral DNA into different sites in chromosomal DNA (*15*). Proviral insertion influences the expression of nearby host genes. The expression of many cellular oncogenes, such as c-myc, c-erb B and int, may be activated as a result of nearby insertion of avian leukemia virus and mouse mammary tumor virus [for a review, see (*15*)]. In other cases, proviral insertion resulted in inactivation of genes. Examples include the α1-collagen I gene (*16*), the p53 gene (*17*), and the dilute gene (*18*). The use of retroviruses as insertion mutagens in cultured mammalian cells and as probes in the molecular cloning of mutant genes requires that proviral insertion be nearly random with respect to cell DNA, that mutant phenotypes be recognized easily, and that insertion-induced mutations occur at a higher frequency than spontaneous mutations. Varmus and his colleagues (*19*) used as the target of mutagenesis a viral src gene present as single copy in a rat fibroblast cell line. We now report insertional mutagenesis at the hypoxanthine phosphoribosyltransferase (hprt) locus (*20*) in cultured F9 EC (*21*) cells after the cells were infected with a variant of the Moloney strain of murine leukemia virus (M-MuLV) (*22*).

Increased mutation frequency. The mouse EC cell lines F9 is derived from the testicular teratocarcinoma OTT 6050 (*21*). This line can be induced to differentiate in vitro into either parietal or visceral endoderm (*23, 24*). This nearly euploid line contains a single X chromosome and presumably a single hprt gene. We chose this X chromosome–linked locus as our mutagenesis model because of its haploidy, because powerful methods exist for selection and counterselection (*25*), and because hprt hybridization probes are available (*26*). If provirus integration occurs at random in chromosomal sites, it should be possible to generate cells in which provirus insertion has occurred within the hprt gene. Insertion into coding sequences should lead to inactivation of the gene, and insertion into flanking or intervening sequences might also have effects on hprt gene expression.

We first investigated the possibility that infection of F9 EC cells with retrovirus would increase the frequency of appearance of hprt-deficient ($hprt^-$) mutations above those occurring spontaneously. Table 1 shows the results of two separate mutagenesis experiments. Infection was achieved by cocultivation of F9 cells with mitomycin C–treated NIH 3T3 cells that produce M-MuLV strain in131SuIII (*22*). Cocultivation for 1 day resulted in five to ten integration sites per F9 cell and for 3 days in 20 to 50 integration sites per cell (*27*).

From amplified libraries of mutagenized cells we have selected for $hprt^-$ mutants by plating cells in media containing 6-thioguanine and 8-azaguanine. In experiment 1, 13 $hprt^-$ clones were recovered from 10^8 infected cells and one from 10^8 uninfected cells. In experiment 2, 70 $hprt^-$ clones were obtained from 10^8 infected F9 cells, but only six were recovered from the same number of uninfected cells. Thus, in both experiments, infection of F9 cells with virus led to a severalfold increase in the frequency of mutation at the hprt locus. For example, in experiment 2, the frequency of induced and spontaneous mutation is 7×10^{-7} and 6×10^{-8}, respectively. Therefore nine of ten $hprt^-$ lines found in the virus-infected cell population should have been generated as a consequence of virus infection.

Altered hprt DNA patterns. To define the events responsible for inactivation of the hprt locus, we analyzed the DNA's of induced and spontaneous $hprt^-$ mutant lines by Southern blotting with a nearly full length hprt complementary DNA (cDNA) probe (*26*). We analyzed 14 virus-infected $hprt^-$ lines and all six of the uninfected $hprt^-$ lines obtained in experiment 2 (Table 1). Figure 1 shows the results obtained with Bam HI–cleaved DNA's from the F9 $hprt^+$ parental line and several $hprt^-$ mutant cell lines. All six of the uninfected $hprt^-$ lines showed a pattern of hybridization identical to that of the F9 parental line (data not shown). In contrast, 4 of the 14

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murine leukemia virus (MLV)-infected hprt$^-$ lines analyzed showed a pattern different from that of the hprt$^+$ parental line (Fig. 1A). Therefore, the DNA structure of the hprt gene has been grossly altered in these lines. These alterations were probably the cause of hprt gene inactivation. In view of the complex structure of the hprt gene (*28*) (Fig. 2B), the remaining lines may also contain structural alterations not detectable by the cDNA probe.

Several types of alterations—namely point mutations, DNA rearrangements, deletions or insertions—could account for the pattern observed with DNA from mutant lines 5, 6, and 9, in which certain wild-type fragments were missing and were replaced by new ones. These possibilities were distinguished by digesting the DNA's from mutant and parental lines with different enzymes and analyzing with probes detecting the 5′ or 3′ part of the hprt gene (Fig. 2). The 5′ hprt probe detects exons 1 to 3, whereas the 3′ hprt probe detects exons 4 to 9 of the hprt gene (*28*) (Fig. 2B).

The 3′ probe showed differences between parental and mutant lines only for line 5 (Fig. 2A). In this line the wild type 9.3-kilobase (kb) Eco RI band was replaced by a 2.8-kb band, the 12.5-kb Bam HI band by a 7.1-kb band, and the 11.4-kb Hind III band by a 7.8-kb band. As judged from the known restriction map of the wild-type hprt gene (*28*) and the provirus (*22*) (shown in Fig. 2B), the generation of those new bands may be best accounted for by a simple model in which a provirus has integrated into the hprt intron between exons 6 and 7. The insertion site would map approximately 2.8 kb upstream from the wild-type Eco RI site located at the end of exon 9 (see arrow in Fig. 2B). The provirus and the hprt gene would be oriented in opposite directions with respect to transcription. As shown below, the isolation and analysis of cloned DNA fragments containing this insertion site confirmed that this interpretation is correct.

Analysis with the 5′ hprt probe showed differences between the parental line and the three mutant lines (Fig. 2A). In line 5, all of the wild-type restriction fragments containing exons 1, 2, or 3 were absent and were replaced by new ones. These data indicate that deletions or rearrangements had affected a large region including sequences located between exons 1 and 3. Therefore, in line 5, provirus insertion in the 3′ region of the gene was followed by alterations of cell sequences in the 5′ region.

In line 6, all of the wild-type restriction fragments containing exons 1 and 2 were missing. The wild-type 1.3-kb Eco RI band and 7.1-kb Hind III band, both of which contain exon 3, were present, whereas the 7.0-kb Bam HI fragment containing exons 2 and 3 was replaced by a 20-kb fragment. This pattern indicates that sequences including exons 1 and 2 have been deleted in line 6. These deletions might also be related to insertion of a provirus into this locus.

In line 9, all of the wild-type restriction fragments containing exon 3, and only these fragments, were altered. Thus the 1.3-kb Eco RI band was replaced by an 0.8-kb band, the 7.0-kb Bam HI band by an 8.0-kb band, and the 7.1-kb Hind III band by an 8.2-kb band. These data indicate that the alterations in line 9 are confined to the 1.3-kb Eco RI fragment containing exon 3. The only simple interpretation of the data is a model in which a provirus has integrated within the intron downstream from exon 3. The site of insertion would be 0.8 kb downstream from the wild-type Eco RI site, which is located just left of exon 3 (see arrow in Fig. 2B). The provirus and the hprt gene would be oriented in opposite directions with respect to transcription.

Isolation of mutant genes. The data presented above indicate that the alterations in hprt sequences in some of the

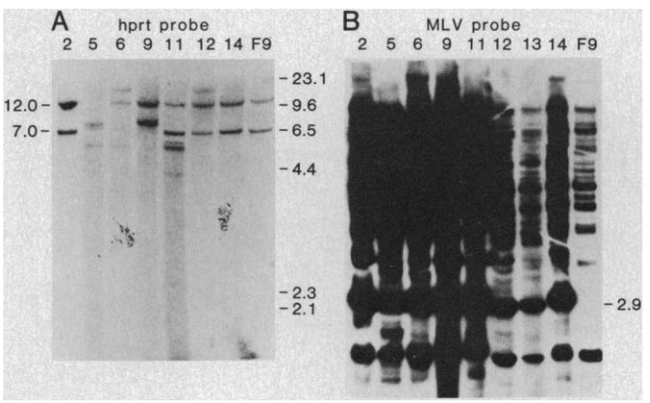

Fig. 1. Bam HI patterns of hprt sequences in virus-infected hprt$^-$ mutant lines. Cellular DNA (10 μg) from parental and mutant lines was digested with Bam HI, subjected to electrophoresis in 0.7 percent agarose gel, transferred to nitrocellulose filters, and hybridized (A) to the hprt cDNA insert from pHPT5 (*26*) or (B) to the plasmid pMLV (*22*). Filters were washed to a final stringency of 0.1× standard saline citrate at 68°C. (A) The hprt cDNA probe detected bands of 12.0 and 7.0 kb and sometimes 4.9 kb in F9 DNA. In mutant lines 5, 6, 9, and 11, hprt bands of altered size were observed. In line 5, the 12.0- and 7.0-kb fragments have been replaced by two new ones. In lines 6 and 9, the 7.0-kb fragment was missing and replaced by a new one. In line 11, new fragments were found in addition to wild-type fragments. (B) The MLV probe detected in F9 DNA many cross-hybridizing bands corresponding to endogenous viruses. However, only infected cell lines contained the 2.9-kb Bam HI fragment diagnostic for the exogenous M-MuLV. A comparison between the intensity of this band with known amounts of cloned M-MuLV DNA (not shown) indicated that these lines contained 20 to 100 proviral copies per genome.

Table 1. Frequency of induced and spontaneous mutation at the hprt locus. F9 cells were grown in hypoxanthine-aminopterin-thymidine (HAT) medium for 1 week before infection. Infection was achieved by plating F9 cells at 1×10^6 to 2×10^6 cells per 10-cm dish onto dishes containing mitomycin C–treated, MLV-in131SuIII (*22*)–producing NIH 3T3 cells (10^6 cells per dish) and cocultivation for various lengths of time. In experiment 1, 50×10^6 F9 cells were plated onto 25 dishes of virus-producing cells. After 1 day of cocultivation, 120×10^6 cells were obtained. This library of mutagenized cells was amplified about tenfold. Selection for hprt$^-$ mutants was carried out as follows: Cells (10^8) from the amplified library were plated at 2×10^6 cells per dish in medium containing 6-thioguanine and 8-azaguanine, each at 30 μg/ml. Colonies were scored after 14 days and ring-cloned. F9 cells in the uninfected control sample were treated in an identical manner but in the absence of MLV-in131SuIII–producing cells. In experiment 2, 10×10^6 F9 cells were plated onto ten dishes of virus-producing cells. After 3 days of cocultivation, 110×10^6 cells were obtained. This library of mutagenized cells was amplified tenfold and selection for hprt$^-$ cells was carried out as described for experiment 1. The average number of proviruses per cell was determined by Southern blotting experiments with the use of DNA from hprt$^-$ clones (Fig. 1B) as well as clones picked randomly after virus infection.

Infection time	Average number of proviruses per cell	Number of hprt$^-$ mutants
	Experiment 1	
Virus-infected (1 day)	5 to 10	13
Mock-infected (1 day)	0	1
	Experiment 2	
Virus-infected (3 days)	20 to 50	70
Mock-infected (3 days)	0	6

mutant lines described were due to insertion of provirus into the hprt locus. In these cases it should be possible to isolate the mutant hprt genes by their linkage to the insertion mutagen. To facilitate this cloning scheme, we have used as the mutagen a variant of M-MuLV that contains the bacterial transfer RNA (tRNA) suppressor gene SuIII (*22*). Thus the tRNA suppressor gene can be used as a marker for the rapid selection of virus-cell DNA junction fragments from genomic DNA libraries constructed in vectors with amber mutations and screened in appropriate bacteria (*22*).

We chose to analyze the four mutant cell lines that show altered patterns of hprt sequences. DNA isolated from the mutant lines 5, 6, 9, and 11 was partially digested with Mbo I and cloned into the Bam HI site of the phage vector Charon 30A. Libraries of 2×10^6 to 5×10^6 independent recombinants were constructed for each mutant cell line and subsequently propagated in LE392, a suppressor-containing ($SupF^+$) host (Table 2). To screen the recombinants for those containing virus cell DNA junction fragments, we plated the libraries onto LG75, a $SupF^-$, lacZ amber host. This $SupF^-$ host will support the growth of only those Charon 30A recombinants that contain a functional suppressor gene. Furthermore, suppression of the amber mutations in the lacZ gene of the host should result in a blue phenotype of

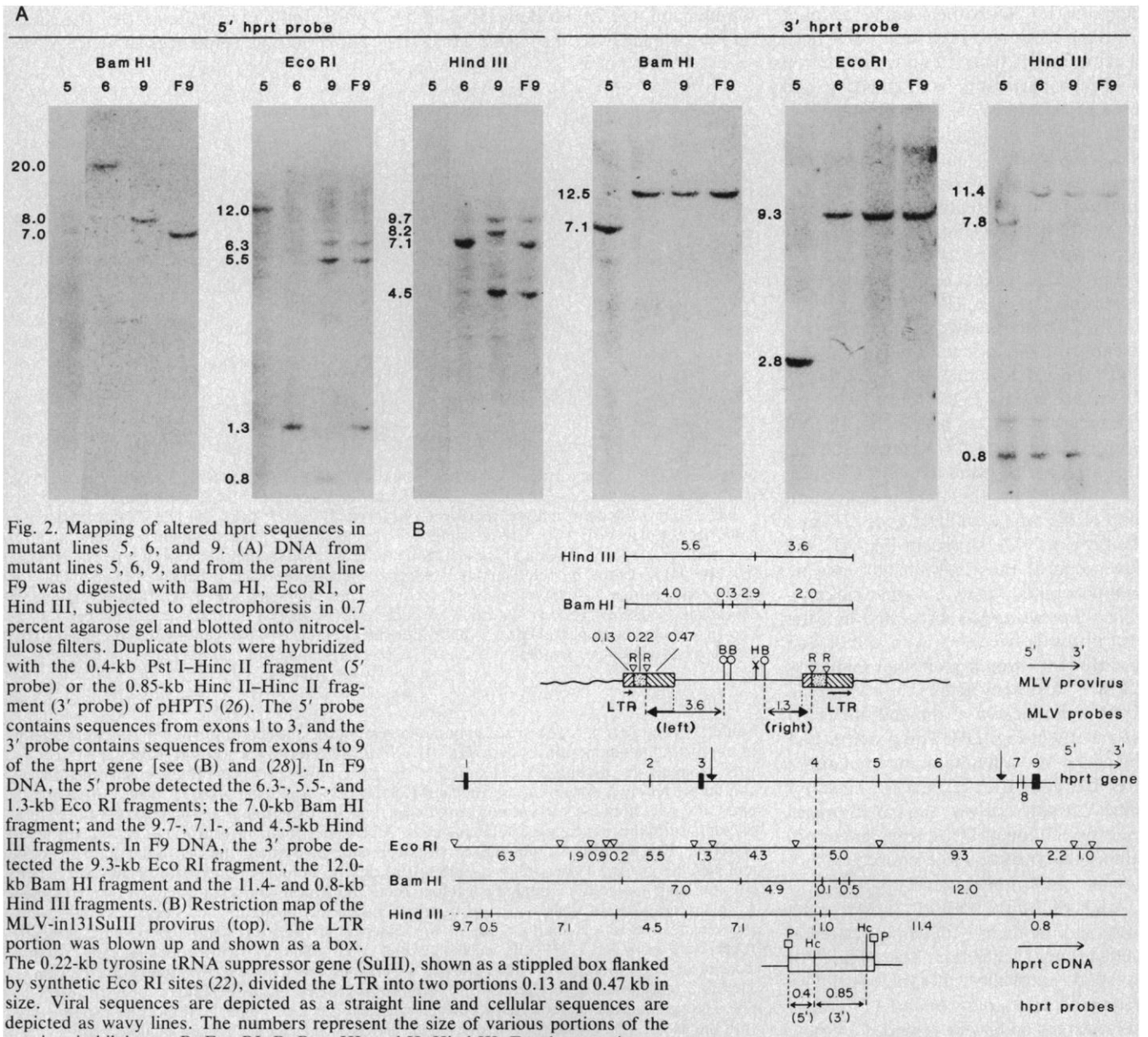

Fig. 2. Mapping of altered hprt sequences in mutant lines 5, 6, and 9. (A) DNA from mutant lines 5, 6, 9, and from the parent line F9 was digested with Bam HI, Eco RI, or Hind III, subjected to electrophoresis in 0.7 percent agarose gel and blotted onto nitrocellulose filters. Duplicate blots were hybridized with the 0.4-kb Pst I–Hinc II fragment (5′ probe) or the 0.85-kb Hinc II–Hinc II fragment (3′ probe) of pHPT5 (*26*). The 5′ probe contains sequences from exons 1 to 3, and the 3′ probe contains sequences from exons 4 to 9 of the hprt gene [see (B) and (*28*)]. In F9 DNA, the 5′ probe detected the 6.3-, 5.5-, and 1.3-kb Eco RI fragments; the 7.0-kb Bam HI fragment; and the 9.7-, 7.1-, and 4.5-kb Hind III fragments. In F9 DNA, the 3′ probe detected the 9.3-kb Eco RI fragment, the 12.0-kb Bam HI fragment and the 11.4- and 0.8-kb Hind III fragments. (B) Restriction map of the MLV-in131SuIII provirus (top). The LTR portion was blown up and shown as a box. The 0.22-kb tyrosine tRNA suppressor gene (SuIII), shown as a stippled box flanked by synthetic Eco RI sites (*22*), divided the LTR into two portions 0.13 and 0.47 kb in size. Viral sequences are depicted as a straight line and cellular sequences are depicted as wavy lines. The numbers represent the size of various portions of the provirus in kilobases. R, Eco RI; B, Bam HI; and H, Hind III. For the experiments shown in Fig. 3, two MLV probes (left and right) were prepared from specfic viral restriction fragments to detect specifically each portion of the proviral LTR. Restriction map of the mouse hprt gene (bottom). The structure of the hprt gene was reproduced from (*28*). A few external restriction sites have been mapped in our studies. The hprt gene contains nine exons (numbered boxes) of total size 1.8 kb, separated by large introns (lines) of total size 32 kb (*28*). In the maps of Eco RI, Bam HI, and Hind III sites, numbers represent the size of the restriction fragments in kilobases. For the studies described here, two hprt cDNA probes (5′ and 3′) were prepared from a Hinc II/Pst I (Hc/P) double digest of the hprt cDNA plasmid pHPT5 (*26*). The 5′ probe contains part of exon 1 (114 nucleotides), exon 2 (107 nucleotides), exon 3 (184 nucleotides), and part of exon 4 (10 nucleotides). The 3′ probe contains part of exon 4 (55 nucleotides), exon 5 (18 nucleotides), exon 6 (83 nucleotides), exon 7 (47 nucleotides), exon 8 (77 nucleotides), and exon 9 (593 nucleotides) (*28*). The two vertical arrows show the sites of MLV-in131SuIII insertion in mutant lines 5 and 9 as deduced from the data shown in (A). In both cases the provirus and the hprt gene were oriented in opposite directions with respect to transcription.

plaques on plates containing X-Gal and IPTG (*29*). As shown in Table 2, between 9 and 35 blue plaques were recovered on LG75 from 10^6 plaques grown on LE392. This frequency is in good agreement with the number of proviruses present in these lines.

To determine whether any of these recombinants contain hprt sequences, we screened the blue plaques by in situ hybridization to a radiolabeled hprt cDNA probe. As shown in Table 2, for two of four cell lines, hprt-containing recombinants were found at a frequency of 6 to 20 hprt-hybridizing plaques per 100 blue plaques. Again this frequency is in good agreement with the number of proviruses found in these cells (20 to 50 provirus copies per diploid genome). Thus, from two of four hprt$^-$ cell lines carrying rearranged hprt sequences it was possible to isolate DNA clones containing both proviral and hprt sequences. In the other lines, the provirus might have inserted into the hprt locus at sequences that cannot be detected by hybridization to the cDNA probe.

Mapping the sites of insertion in cloned DNA. We determined precisely the site of insertion of MLV-in131SuIII in the hprt gene of one mutant line. Four recombinants isolated from cell line 5 were analyzed by restriction mapping and blot hybridization (Fig. 3). In our isolation protocol we had selected and screened for recombinants carrying a functional SuIII gene and some hprt exon sequences. Therefore, we predicted that Eco RI digestion of the recombinants should excise the 0.2-kb SuIII gene from the provirus long terminal repeat (LTR) and divide the LTR into two portions, the left one 0.13 kb long and the right one 0.47 kb long (Fig. 2B). In recombinants containing the provirus 5′ LTR, the left portion should be linked to cell DNA and the right portion linked to viral DNA. The reverse is true in recombinants containing the provirus 3′ LTR.

By using two MLV hybridization probes that can distinguish between this left and right portion of the LTR (shown in Fig. 2B), we found in all four recombinants a 2.8-kb Eco RI fragment containing 3′ hprt sequences and the left portion of the LTR (Fig. 3A). Therefore this 2.8-kb fragment contained the junction between the provirus 5′ LTR and cell DNA that included some hprt exon sequences. From the data presented in Fig. 3A, we deduced the Eco RI maps of the recombinants shown in Fig. 3B. Hind III cut the 2.8-kb Eco RI hybridizing band once to give rise to 2.35- and 0.45-kb hybridizing bands (data not shown). We concluded that the provirus had inserted at about 2.35 kb upstream from the Hind III site within exon 9 or 2.8 kb upstream from the Eco RI site at the 3′ end of exon 9 (Fig. 3C). This site is in the intron between exons 6 and 7. The provirus and the hprt gene in cell line 5 are oriented in opposite directions with respect to transcription. The data obtained with cloned DNA and genomic DNA from mutant line 5 are thus in complete agreement.

Discussion. Our studies demonstrate the successful use of retroviruses as insertion mutagens in cultured mammalian cells and as probes in the recovery of genes encoding the mutant phenotypes. As our model, we studied the X-linked haploid locus encoding hypoxanthine phosphoribosyltransferase. We showed that infection of EC cells with retrovirus caused a severalfold increase in the frequency of occurrence of hprt$^-$ mutations. From mutant cells selected for resistance to thioguanine and azaguanine, we isolated mutant hprt genes by using the inserting virus as a probe. The cloning scheme was simplified by the use of a rescuable variant of M-MuLV carrying a bacterial suppressor tRNA gene.

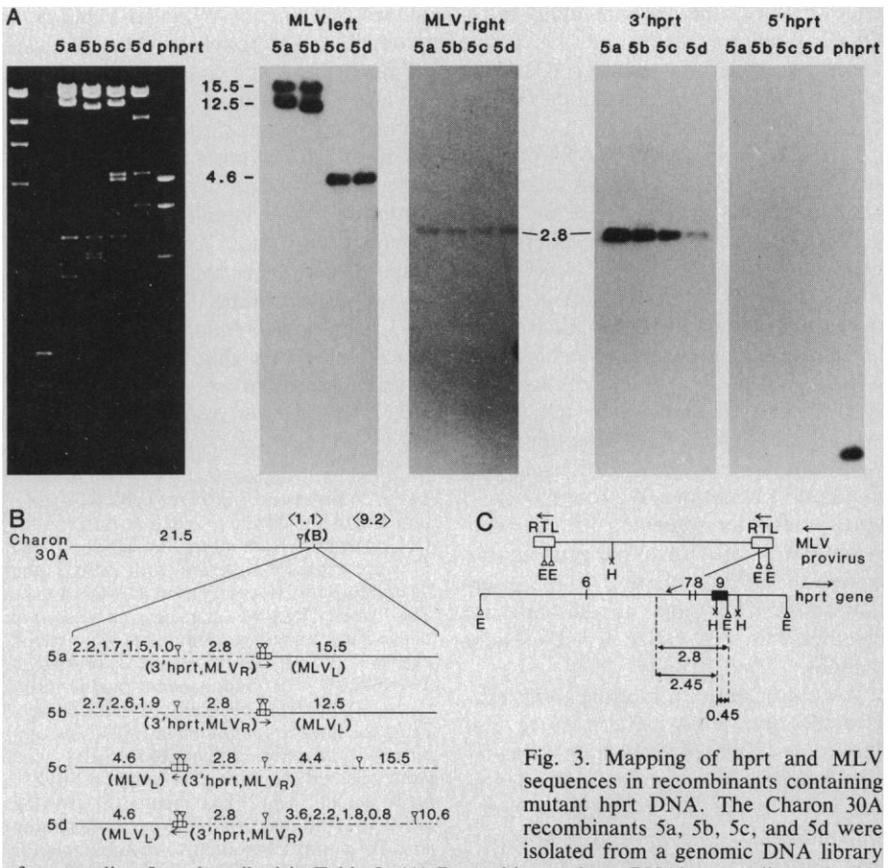

Fig. 3. Mapping of hprt and MLV sequences in recombinants containing mutant hprt DNA. The Charon 30A recombinants 5a, 5b, 5c, and 5d were isolated from a genomic DNA library of mutant line 5 as described in Table 2. (A) Recombinant phage DNA's were digested with Eco RI and subjected to electrophoresis on a 1.2 percent agarose gel. Four identical nitrocellulose blots were prepared and hybridized to the different viral and hprt probes (shown in Fig. 2B). The 5′ hprt probe and the MLV right probe hybridized to a 2.8-kb fragment in all recombinants. The MLV left probe hybridized to two bands in recombinants 5a and 5b. This was due to aggregation of the 15.5- or the 12.5-kb fragment to the 21.5-kb fragment via annealing of the phage cos sites. (B) The deduced Eco RI map of the Charon 30A recombinants. Eco RI sites are represented by open triangles. The Bam HI site in Charon 30A which was lost upon ligation to Mbo I sites is designated as B. The numbers represented the size in kilobases of the Eco RI restriction fragments. The LTR is depicted as a box, and its transcription direction is depicted by an arrow. Cellular DNA and viral DNA are represented as dashed lines and solid lines, respectively. Positive hybridization to a probe is shown in parenthesis. All recombinants contained a 2.8-kb fragment that hybridized to the hprt 3′ probe and the MLV right probe (therefore containing the left portion of the LTR). On one side of the 2.8-kb fragment was the 0.22-kb SuIII gene fragment (not detected in our gel system) and a fragment that hybridized to the MLV left probe (therefore containing the right portion of the LTR). The size of this fragment varied in the different recombinants because Mbo I cut at different sites in the viral sequences. On the other side of the 2.8-kb fragment were Eco RI fragments containing cell DNA. These fragments are different in different recombinants and their order has not been mapped. Since the Mbo I partial digest of cellular DNA had not been selected for size before cloning, the recombinants may fortuitously contain more than one Mbo I partial fragment of cell DNA. (C) The site of proviral insertion in mutant line 5 as deduced from restriction mapping data. The provirus was integrated into the intron between exons 6 and 7 of the hprt gene and was in the direction opposite the hprt gene.

In cell populations containing 20 to 50 proviruses per genome, the mutation frequency at the hprt locus was 7×10^{-7} as compared to a frequency of 6×10^{-8} in uninfected cells. In studies of inactivation of a single-copy viral src gene in a virally transformed rat cell line by M-MuLV superinfection, Varmus *et al.* (*19*) found that the frequency of insertion-induced mutations was 1 to 10 percent that of spontaneous mutations. In this case, using the virus as a probe to isolate mutant genes would have been impractical. The important difference between frequencies of mutations found in the two systems might reflect intrinsic differences between the experimentally introduced viral src locus and the native cellular hprt locus or between B31 fibroblast cells and F9 embryonal carcinoma cells.

The hprt gene contains 1.8 kb of exon sequences and 32 kb of intron sequences (*28*). Therefore the target size for insertion mutagenesis to an hprt$^-$ phenotype lies somewhere between 1,800 and 34,000 nucleotides. If the sites of proviral integration are randomly distributed in cell DNA, the screening of a library of 2×10^9 to 5×10^9 independent insertion sites should have covered the entire mouse genome (3×10^9 nucleotides) and yielded 1,800 to 34,000 insertions leading to a hprt$^-$ phenotype. We found only 70 hprt$^-$ lines after screening 10^8 cells carrying 20 to 50 proviruses per genome (the equivalent of 2×10^9 to 5×10^9 insertion sites). This suggests that proviral insertion into cell DNA is not entirely random.

We used Southern blotting to investigate the hprt DNA pattern of several hprt$^-$ lines isolated from both virus-infected and uninfected EC cell populations. All of the six spontaneous hprt$^-$ mutant lines studied showed an hprt pattern identical to that of the wild-type, parental line. Therefore in spontaneous hprt$^-$ EC mutants, small lesions in the hprt gene (base substitutions and frameshifts) rather than gross alterations (deletions, insertions, or rearrangements) are the responsible mutational events. A significant number (4 of 14) of virus-infected, hprt$^-$ mutant lines studied showed an hprt pattern different from that of the wild-type parental line. Further analysis demonstrated clearly the insertion of provirus into the hprt gene in two lines. In each of these two lines, the site of provirus insertion was mapped to a different intron of the gene. This finding extends previous observations (*16–19*) and indicates that the target size for insertion mutagenesis by retrovirus is not restricted to protein and messenger RNA coding sequences but includes intron sequences as well.

This method of mutagenesis should permit the isolation of any mammalian gene, the insertional activation or inactivation of which is inducible over the spontaneous background and produces cellular alterations that can be screened or selected for. The mutagenesis results we obtained with the hprt gene should be applicable to all haploid genes as well as to diploid genes if correction is made for diploidy. In mammalian cells, functional haploid genes include genes on the X and Y chromosomes as well as functional immunoglobulin genes in lymphocytes or alleles that encode distinguishable gene products in heterozygous cells. The rate of mutagenesis at a diploid locus is usually 1 to 2 percent of that at a haploid locus. This means in practice that about 50 to 100 times more cells have to be screened to obtain the same number of mutants. For example, in preliminary attempts to study diploid genes, we found that adenine phosphoribosyltransferase–deficient (aprt$^-$) mutants occur 2 to 3 percent as frequently as hprt$^-$ mutants. Insertions that inactivate genes usually represent recessive mutations (*16, 18*). However, retroviral insertions can also activate genes and therefore represent dominant mutations (*15*). It may therefore be feasible to induce dominant mutations in EC cells by retroviral insertions.

The method we have described for mutagenesis and cloning of genes might prove useful for the genetic dissection of various cell phenotypes. These phenotypes might include cell surface antigenicity, growth control, differentiation, or embryonic development.

Table 2. Isolation of proviral cell DNA junction fragments by means of the SuIII gene. DNA prepared from mutant lines 5, 6, 9, and 11 were partially digested with Mbo I and ligated to Bam HI–cut Charon 30A DNA (*30*). The ligated DNA was packaged in vitro into phage particles and recombinants were plated onto LE 392, a SupF$^+$ strain. Libraries of 2×10^6 to 7×10^6 independent plaque-forming units (pfu) were obtained for each cell line. From the libraries amplified in LE392, recombinants containing a functional SuIII gene were selected by plating on LG75, a SupF$^-$, lacZ amber host. Exponentially growing LG75 (0.2 ml) in Luria broth were incubated with 10^6 pfu of Charon 30A recombinants for 20 minutes at 37°C. After addition of 80 μl of X-Gal (20 mg/ml), 20 μl of 100 m*M* IPTG, and 3 ml of soft agar, the mixture was plated onto one 10-cm bottom agar plate. Blue plaques were scored on the next day. Numerous colorless plaques (100 to 1000) were also found, probably as a result of recombination between phage DNA present in the packaging extracts. From recombinants carrying a functional SuIII gene (blue plaque phenotype), those that also contain hprt cDNA sequences were screened by in situ plaque hybridization to the radiolabeled Pst I insert probe from the plasmid pHPT5 (*26*).

Mutant hprt$^-$ line	LE392 (pfu)	LG75 (blue pfu)	Number of hprt-hybridizing clones
5	1×10^6	25	5
6	1×10^6	9	0
9	1×10^6	35	2
11	1×10^6	22	0

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