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Genetics

Retrovirus long terminal repeats activate expression of coding sequences for the herpes simplex virus thymidine kinase gene

(gene transfer/recombinant DNA/transcriptional promoters)

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ABSTRACT **In this study, we demonstrate that the long terminal repeats (LTRs) of a murine retrovirus can activate expression of heterologous gene coding sequences from which a functional promoter region has been deleted. Recombinant plasmid clones were obtained that contained both cloned fragments of Friend spleen focus-forming virus (SFFV) DNA and the herpes simplex virus (HSV) thymidine kinase (TK; ATP:thymidine 5′-phosphotransferase, EC 2.7.1.21) gene (*tk*). The effects of the LTR on *tk* expression were determined by constructing clones containing *tk* coding sequences with or without 5′ sequences necessary for the initiation of transcription, inserted either 200 or 1200 base pairs downstream from the SFFV 5′ LTR. The expression of the HSV TK protein by these clones was tested by gene transfer of the clones into TK^- mouse cells and assay of TK enzyme activity in TK^+ transformants. These experiments demonstrate that: (*i*) the SFFV 5′ LTR activates expression of *tk* coding sequences when these sequences are inserted 200 base pairs downstream from, and in the same orientation as, the LTR; (*ii*) *tk* is not activated when placed 1200 base pairs downstream from, and in the same orientation as, the LTR or when *tk* is inserted in either site in the opposite orientation as the LTR; (*iii*) the SFFV 5′ LTR does not interfere with *in vivo* expression of *tk* when it is flanked by homologous 5′ promoter sequences. The implication of these observations for retrovirus oncogenesis and animal cell genetics is discussed.**

Retrovirus genomes share a number of similarities in structure with transposable genetic elements (1–4). In particular, the integrated retrovirus provirus contains long terminal repeats (LTRs) at the junction between the host and viral genomes. These LTRs, which are 500–600 base pairs (bp) in length, are formed during reverse transcription and DNA synthesis of the provirus (5, 6), and are composed of unique as well as repeated sequences, derived from the 5′ and 3′ ends of the genomic viral RNA. Nucleotide sequence analysis of the termini of integrated retroviruses has revealed a number of similarities with sequences that form the corresponding termini of transposable elements found in prokaryotes and certain lower eukaryotes. These include a short (3- to 6-bp) direct repeat of host DNA at the junction between the provirus and host DNA (7, 8), a short (11-bp) inverted repeat at the termini of each LTR (1–4, 7, 8), and the presence of transcriptional control signals, including regions implicated in the initiation and termination of transcription (9, 10). Thus, LTRs appear to be involved in a number of key viral functions, including the replication, integration, and expression of retrovirus genomes.

LTRs also have a central role in the induction of malignant transformation by retroviruses. These viruses can be classified into two groups on the basis of the speed and efficiency with which they induce neoplastic transformation. The chronic viruses induce leukemia or leukosis in susceptible animals with low efficiency and after a long latent period (4–12 months). In contrast, the acute viruses induce sarcomas or acute leukemias with high efficiency and short latent periods (1–4 weeks). Neoplastic transformation by both groups of viruses may result from the activation of cellular genetic sequences by LTRs. For the acute viruses, these cellular sequences, or potential oncogenes (11, 12), are transduced as a stable part of the viral genome; hence, the synthesis, integration, and transcription of these sequences is controlled, like virion structural genes, by the viral LTRs. At least 10 different transforming genes have been identified among the avian and mammalian acute retroviruses analyzed, and in each case, these sequences have also been detected in the genome of normal cells (11, 12). In contrast, the genomes of the chronic leukemia viruses do not appear to include unique sequences derived from normal cell DNA. However, analysis of proviral DNA integration sites, as well as RNA transcripts, in tumor cells induced by avian leukosis virus (ALV) suggests that the induction of lymphoid tumors by this virus results from its integration adjacent to specific cellular sequences corresponding to the transforming region of the avian acute leukemia virus, MC-29 (13–16). Evidence has recently been presented indicating that this integration event then results in the enhanced expression of these adjacent cellular sequences (16).

These observations suggest that retrovirus LTRs can activate potential oncogenes that are part of the host or retrovirus genome. However, it is not evident whether LTRs can also activate expression of heterologous DNA sequences that have not been found associated as part of a retrovirus genome. In this study, we have utilized gene transfer of the thymidine kinase (TK; ATP:thymidine 5′-phosphotransferase, EC 2.7.1.21) gene (*tk*) of herpes simplex virus type I (HSV-1) to show that the cloned LTR of the polycythemia-inducing strain of Friend spleen focus-forming virus ($SFFV_P$) can activate expression of the *tk* coding sequence from which its own promoter region has been deleted.

MATERIALS AND METHODS

Cell Culture. The recipient LTA cell line used in the eukaryotic transformation experiments was an adenine phosphoribosyltransferase-negative ($APRT^-$) derivative of TK^- mouse L cells (17), derived by R. Hughes and P. Plagemann. The LTA cells were maintained in α minimal essential medium (18) supplemented with 10% fetal calf serum. TK^+ cells were selected in the same medium supplemented with 0.1 mM hypoxanthine/1.0 μM aminopterin/40 μM thymidine (HAT).

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Abbreviations: LTR, long terminal repeat; TK, thymidine kinase; bp, base pair(s); kb, kilobase pair(s); HAT, hypoxanthine/aminopterin/thymidine; ALV, avian leukosis virus; $SFFV_P$, polycythemia-inducing strain of spleen focus-forming virus; HSV-1, herpes simplex virus type I; Mo-MSV, Moloney strain of murine sarcoma virus.

Bacterial Strains, Plasmids, and Recombinant Phage. The *Escherichia coli* K-12 derivative HB101 (19) was obtained from H. Temin. *E. coli* LE392 (20) was obtained from P. Leder. The *E. coli* LE392 derivative LE576 (21), carrying the plasmid pxl, was obtained from J. Smiley. The construction and characterization of the recombinant phage λSFFV$_p$502 has been described elsewhere (22).

Enzymes. Restriction endonuclease digestions were performed using 1–2 units of enzyme per μg of DNA for 2–6 hr under the conditions specified by the supplier (Boehringer Mannheim or Bethesda Research Laboratories). Phage T4 DNA ligase was obtained from New England BioLabs and all reactions were carried out overnight at 15°C in the recommended ligation buffer containing 1000 units of enzyme per μg of DNA and DNA at a concentration of 20–30 μg/ml. The ligation mixture was then heated to 65°C and samples were used directly to transfect bacteria. Bacterial alkaline phosphatase was obtained from Bethesda Research Laboratories, and all reactions were carried out in 10 mM Tris·HCl (pH 8) at 37°C for 2 hr.

Bacterial Transformation and Construction of Recombinant Plasmids. *E. coli* LE392 was used for transformation with the plasmid pYY508 and *E. coli* HB101 was used for all other transformations (23).

For cloning, the *Bam*HI *tk*, *Bgl* II/*Bam*HI *tk*, and *Eco*RI SFFV$_p$502 DNA fragments were isolated by preparative gel electrophoresis and electroelution (24). To construct the plasmid pYY508, 1 μg of pBR322 was digested with the restriction enzymes *Eco*RI, *Bam*HI, and *Hin*dIII and 1 μg of the 7.4-kilobase pair (kb) *Eco*RI SFFV$_p$502 fragment was digested with *Bam*HI. After heat inactivation, equal amounts (500 ng) of the digested pBR322 DNA and digested SFFV$_p$502 DNA were ligated. pAJ2 was constructed by ligating equal amounts (600 ng) of phosphatase-treated *Bam*HI-digested pBR322 and the *Bgl* II/*Bam*HI *tk* fragment. The eight hybrid clones containing a fragment of *tk* inserted into pYY508 were constructed by ligating equal amounts (600 ng) of either phosphatase-treated *Bgl* II- or *Bam*HI-digested pYY508 with the *Bam*HI *tk* or *Bgl* II/*Bam*HI *tk* fragment.

Eukaryotic Cell Transformation. LTA cells (7×10^5) seeded in 100-mm Petri dishes were transfected with plasmid DNA mixed with LTA carrier DNA as described (25) with modifications (26). Cultures were switched to HAT medium 40 hr later. Two weeks later transformed colonies were fixed in 10% formalin and stained in 0.1% methylene blue.

Assay of TK Activity. The method used was a modification of the technique described previously (27). Sonicated cell extracts were centrifuged at $130,000 \times g$ for 5 min and the supernatant was assayed for both TK activity (27) and protein concentration. Rabbit antiserum against HSV-1 TK was kindly provided by S. Girvitz at McMaster University. Immune or normal rabbit serum was mixed with a 25-fold excess volume of reaction mixture lacking [^{3}H]thymidine and incubated at 4°C for 30 min before the TK activity was measured at 37°C in the presence of 2 μCi of [^{3}H]thymidine (specific activity 51 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels) for 1 hr. One unit of TK activity is defined as the amount of enzyme that converts 1 nmol of thymidine into thymidine monophosphate per min.

RESULTS

Construction of Recombinant Plasmids Containing the SFFV$_p$ LTR and HSV-1 TK Coding Sequences. To determine whether a retrovirus LTR could activate expression of heterologous coding sequences, we constructed recombinant clones containing the 5′ LTR of Friend SFFV$_p$ and the HSV-1 *tk* gene. As described in *Materials and Methods* and Fig. 1, the 5′ end

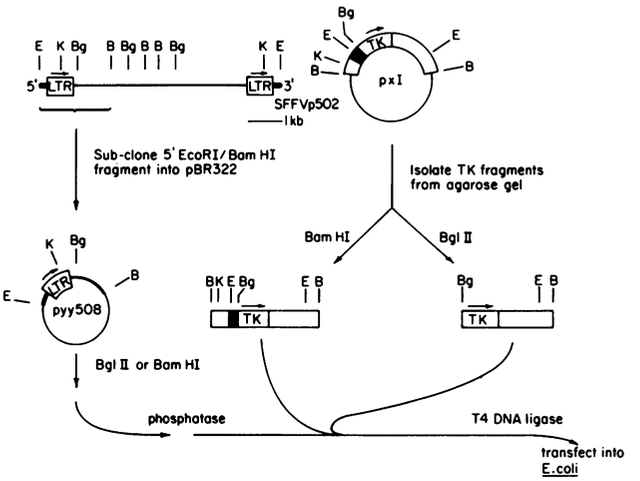
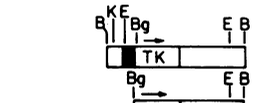
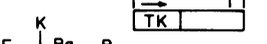
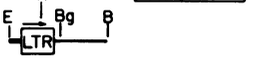
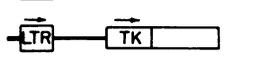
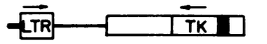
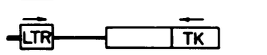
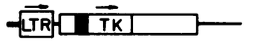
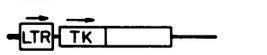
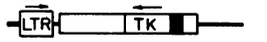
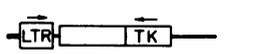


FIG. 1. Strategy for the construction of pLTR–TK recombinant plasmids. The diagram is drawn approximately to scale. SFFV$_p$502 sequences are indicated by the line of intermediate thickness, rat flanking sequences by thick lines, and pBR322 sequences by a thin line. The *Bam*HI *tk* fragment is indicated by the large open box surrounding the smaller box marked TK. The smaller TK box indicates the *tk* coding sequences and the adjacent filled-in box indicates the *tk* promoter region. The arrows indicate the direction of transcription. Restriction endonuclease sites: B, *Bam*HI; Bg, *Bgl* II; E, *Eco*RI; K, *Kpn* I.

fragment of SFFV$_p$502, extending from the *Eco*RI site in the rat flanking sequences to the *Bam*HI site located 1.2 kb 3′ of the 5′ LTR, was subcloned into *Eco*RI/*Bam*HI-digested pBR322 (pYY508; Fig. 1). This fragment has one *Bgl* II site approximately 200 nucleotides 3′ of the 5′ LTR. The *Bam*HI frag-

Table 1. TK transforming activity of recombinant pLTR–TK plasmids

Plasmid	Schematic of plasmid	Transformation efficiency, no. TK$^+$ colonies/pmol plasmid	
pxl		13,000	(6)
pAJ2		0	(5)
pYY508		0	(3)
pAJ11		20,000	(3)
pAJ21		0	(5)
pAJ12		12,000	(5)
pAJ22		0	(6)
pAJ13		13,000	(6)
pAJ23		14,000	(5)
pAJ14		22,000	(5)
pAJ24		0	(6)

Approximately 100 ng of each plasmid DNA was mixed with 20 μg of carrier DNA and used to transform LTA cells. The numbers in parentheses are the number of dishes used to calculate the transforming efficiency. The diagrams are drawn approximately to scale, using the symbols indicated in the legend to Fig. 1.

ment of HSV-1 containing the *tk* gene has been cloned by Enquist *et al.* (21) in pBR322 (px1). DNA transfer experiments (21, 28) and DNA sequence analysis data (29, 30) indicate that this fragment contains the homologous promoter region for this gene. DNA sequence analysis data and mRNA mapping of the *tk* gene (29, 30) indicate that there is a *Bgl* II site in the leader mRNA sequence of this gene. Thus, digestion of px1 with *Bgl* II and *Bam*HI produces a 2.8-kb fragment containing the intact coding sequence of *tk* but lacking 5′ sequences required for expression of the *tk* gene in mammalian cells. This 2.8-kb *Bgl* II/*Bam*HI *tk* fragment was isolated and inserted into the *Bam*HI site of pBR322 (pAJ2; Table 1).

The eight pLTR–TK clones were constructed as described in *Materials and Methods* by ligating linearized pYY508 to either the *Bam*HI or *Bgl* II/*Bam*HI *tk* fragment and transfecting this DNA into the bacterium HB101 (see Fig. 1). Plasmids from ampicillin-resistant colonies were isolated by a rapid boiling method (31) and screened by restriction enzyme analysis for the insertion and orientation of *tk* fragments into pYY508 at either the *Bam*HI or the *Bgl* II site. To determine the orientation of *tk* in the eight possible pLTR–TK clones and pAJ2, these clones were digested with *Kpn* I or *Eco*RI and analyzed by gel electrophoresis (Fig. 2*a*) and the Southern blot technique (32) (Fig. 2*b*) using a nick-translated 2.3-kb *Bgl* II/*Eco*RI *tk* fragment as probe. From Fig. 1 and Table 1 it can be seen that digestion of clones pAJ11, -12, -13, and -14 with *Kpn* I and pAJ21, -22, -23, -24, and -2 with *Eco*RI gives restriction fragment patterns that directly determine the orientation of *tk* in the clones. Because digestion of pAJ13 with *Kpn* I produces a large fragment of 9.25 kb and a smaller fragment of 0.35 kb that cannot be resolved on a 1% agarose gel, this clone was therefore also digested with *Eco*RI to confirm its structure. Similarly, pAJ21, when cut with *Eco*RI, gives two bands of almost identical length, 4.5 kb, which cannot be resolved. This clone was, therefore, also digested with *Eco*RI and *Bgl* II to confirm its structure. pYY508 was digested with *Bam*HI and *Eco*RI to demonstrate the presence of a 2.2-kb insert that does not hybridize to the *tk* probe (Fig. 2 *a* and *b*).

Transforming Activity of Cloned pLTR–TK Recombinants. The transformation efficiency of each of the eight pLTR–TK recombinant plasmids was tested by transferring the plasmids into TK$^-$ L cells (LTA) by the calcium phosphate technique (26) and selecting for TK$^+$ transformants in HAT medium. Table 1 shows the combined results of two DNA transfer experiments. DNA transfer of the intact *Bam*HI *tk* fragment transformed TK$^-$ mouse cells to a TK$^+$ phenotype with a high frequency whether the *tk* fragment was inserted into pBR322 alone (px1) or inserted in either orientation into the *Bgl* II or *Bam*HI sites of pYY508 (pAJ11, -12, -13, -14). In contrast, the plasmid pAJ2, containing only the *tk* coding sequence and 3′ flanking sequences, did not transform LTA cells to a TK$^+$ phenotype. Similar observations have been made with *Bgl* II-digested px1 (21). Recombinant plasmids containing the *Bgl* II/*Bam*HI *tk* fragment inserted into either the *Bgl* II or *Bam*HI site of pYY508 in the orientation of transcription opposite to that of the LTR (AJ22, AJ24) did not transform LTA cells to a TK$^+$ phenotype. However, the plasmid pAJ23, containing the *Bgl* II/*Bam*HI *tk* fragment inserted into the *Bgl* II site of pYY508 in the same orientation as the LTR, transformed LTA cells at a frequency similar to that observed with the plasmids containing the entire *Bam*HI *tk* fragment. Interestingly, DNA transfer of pAJ21, a plasmid containing the *Bgl* II/*Bam*HI *tk* fragment inserted into the *Bam*HI site of pYY508 in the same orientation as the LTR, did not result in any TK$^+$ transformants.

Mass Ligation and Cotransfer of the SFFV$_p$502 LTRs and *tk* Coding Sequences. The experiments presented above demonstrate that cloned DNA molecules containing the *Eco*RI/*Bgl* II 5′ LTR fragment of SFFV$_p$502 5′ to, and in the same orientation as, the *tk* coding sequences can promote the transforming activity of *tk* coding sequences. It was therefore of interest to determine whether the SFFV$_p$502 LTR could also activate expression of *tk* coding sequences by mass ligating or mixing these sequences prior to transfection into TK$^-$ mouse L cells. The *Bgl* II/*Bam*HI *tk* fragment was mass ligated *in vitro*, as described in *Materials and Methods*, either to an approximately equivalent molar quantity of *Bgl* II-digested pYY508 or to a 1/3rd or 1/6th molar quantity of *Bgl* II-digested SFFV$_p$502. For cotransfer experiments, the same molar ratios of DNA were simply mixed and used for transformation. It can be seen from Table 2 that transformation with either 150 or 450

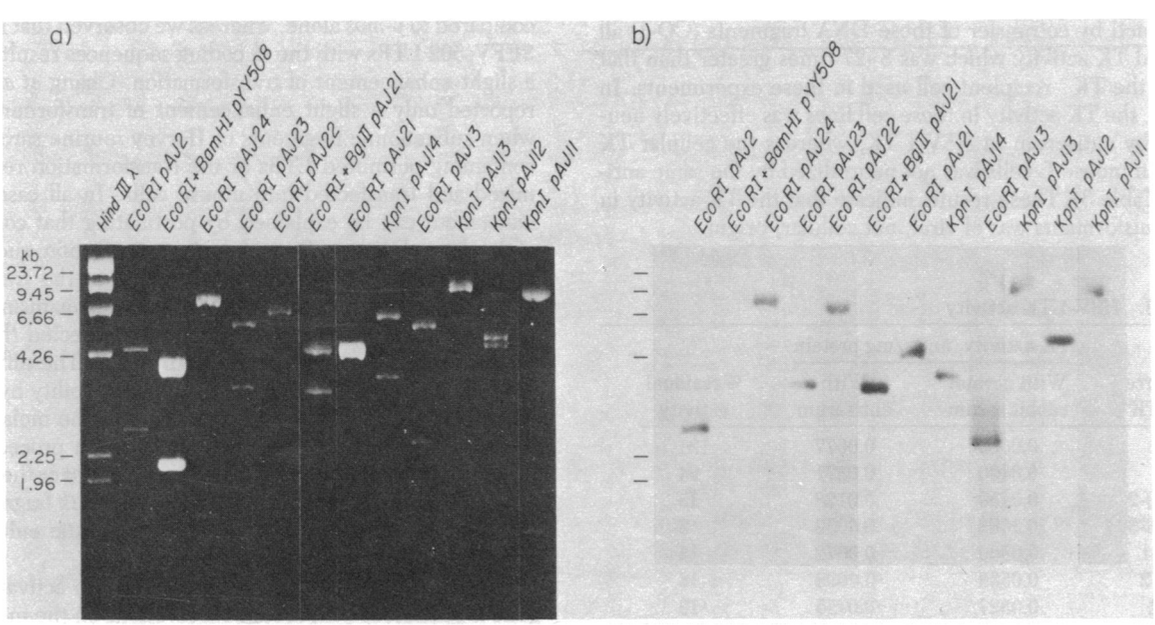


FIG. 2. Analysis of the *tk* sequences present in pLTR–TK clones. The various recombinant plasmid DNA clones were cleaved by the restriction enzymes indicated, electrophoresed on a 1% agarose gel, stained with ethidium bromide, and visualized with ultraviolet light (*a*) and by the technique of Southern (32) (*b*) with a ^{32}P-labeled 2.3-kb *Bgl* II/*Bam*HI *tk* fragment probe.

Table 2. Enhanced transformation of *tk* coding sequences mass ligated to Friend LTRs

Bgl II/*Bam*HI *tk*, ng	*Bgl* II-digested $SFFV_P502$, ng	*Bgl* II-digested pYY508, ng	Ligation	Transformation efficiency, no. TK^+ colonies/pmol *Bgl* II/*Bam*HI fragment*
450			−	0 (0/3)
450	200		−	2.7 (2/3)
450	200		+	8 (6/3)
150				0 (0/3)
150		300	−	4.1 (1/3)
150		300	+	81.3 (20/3)
250	250		+	80.3 (33/3)

The quantities of DNA indicated were mixed and ligated by using T4 ligase (where indicated by +) under standard conditions. Carrier DNA (20 μg) was then added to each sample and this was used to transform LTA cells.

* The number in parentheses is the number of TK^+ colonies per number of plates scored.

ng of the *Bgl* II/*Bam*HI *tk* fragment per plate did not result in any TK^+ transformants, in agreement with previous results (21) and the observation in Table 1 with the cloned *Bgl* II/*Bam*HI *tk* fragment. When this fragment was mass ligated to either *Bgl* II-digested $SFFV_P502$ or pYY508, there was a significant enhancement of TK^+ transformation. Results of the cotransfer experiments indicated that simply mixing the *Bgl* II/*Bam*HI *tk* fragment with $SFFV_P502$ LTRs resulted in a low number of TK^+ transformants.

TK^+ Transformed Cells Express HSV-1 TK Activity. To determine the origin of the TK activity present in the HAT-resistant transformants described in Tables 1 and 2, we made use of the fact that the viral TK activity can be distinguished antigenically from the cellular TK enzyme by antiserum raised against HSV-1 TK (33). The experiments shown in Table 3 indicate that the TK activity of two cell lines transformed by the plasmid pAJ23 (AJ23-2, AJ23-3), two cell lines transformed by ligated DNA containing the *Bgl* II/*Bam*HI *tk* fragment and *Bgl* II-digested $SFFV_P502$ DNA (LIG-1, LIG-2), and one cell line transformed by cotransfer of these DNA fragments (CO-1) all contained TK activity which was 8–27 times greater than that of LTA, the TK^- recipient cell used in these experiments. In addition, the TK activity in these cell lines was effectively neutralized by antiserum to HSV-1 TK, whereas the cellular TK activity in mouse L cells was not neutralized by the same antiserum (Table 3). These results indicate that the TK activity in these transformants was of viral, not cellular, origin.

Table 3. HSV-1 TK activity

Source of TK	TK activity, units/mg protein: With normal rabbit serum	With antiserum	% residual activity
LTA	0.0063	0.0077	121
NIH	0.0400	0.0371	94
AJ23-2	0.0850	0.0128	15
AJ23-3	0.1680	0.0052	3
LIG-1	0.0500	0.0072	14
LIG-2	0.0533	0.0098	18
CO-1	0.0827	0.0155	19

Supernatants (130,000 × *g*) of homogenates from the above cell lines were mixed with normal rabbit serum or antiserum to purified HSV-1 TK, and TK activity was assayed.

DISCUSSION

From the experiments presented in this study we conclude that the LTR of $SFFV_P502$ can functionally activate the continuous expression of heterologous *tk* coding sequences from which transcription initiation signals have been deleted. In addition, these experiments indicate that a retrovirus LTR does not interfere with the *in vivo* expression of DNA segments that contain, along with the *tk* coding sequences, homologous 5′ flanking sequences that include the presumptive promoter region of the gene.

A number of observations suggest that the Friend virus LTR stimulates *tk* expression by transcriptional activation. First, the *Bgl* II/*Bam*HI DNA segment containing *tk* coding sequences was activated only when it was in the same transcriptional polarity as the LTR, whereas the *Bam*HI *tk* fragment that contains its own transcription initiation sequences was expressed independent of its polarity relative to the LTR. Second, the *tk* coding sequences were activated only when located 200 bp downstream from the LTR and were inactive when inserted 1.2 kb 3′ to the LTR. In contrast, the *Bam*HI *tk* fragment was equally functional when inserted into either downstream site. Third, the transformation efficiency of the *Bam*HI *tk* fragment was not enhanced when cloned near the $SFFV_P502$ 5′ LTR.

Expression of the *Bgl* II/*Bam*HI *tk* fragment depended not only on its orientation but also on its location relative to the LTR. It is possible that the presence of an additional 1 kb of retroviral sequences between the LTR and *tk* coding sequence interferes with either the transcription or translation of these sequences. Alternatively, a functionally inactive polyprotein, consisting of the *tk* protein and retroviral sequences encoded by the 5′ region of $SFFV_P$, may be synthesized.

Our results confirm and extend previous observations by others (34, 35) that ligation or cotransfer of cloned LTR sequences with cloned fragments of the transforming gene of Moloney murine sarcoma virus (Mo-MSV) (*v-mos*) or its cellular counterpart (*c-mos*) increased transformation efficiency in transfection experiments similar to those reported here. In contrast to the present studies with *tk*, Blair *et al.* (34) reported that mixing the Mo-MSV LTR with *v-mos* sequences prior to transfection resulted in a several-hundredfold stimulation of activity compared to *v-mos* alone, whereas we observed that mixing the $SFFV_P502$ LTRs with the *tk* coding sequences resulted in only a slight enhancement of transformation. Chang *et al.* (36) also reported only a slight enhancement of transforming activity when subgenomic fragments of Harvey murine sarcoma virus containing permuted LTRs or the transformation region were mixed and transfected into mouse cells. In all cases the enhancement can be explained by postulating that concatamers of the donor DNA are formed during transfection, such that the LTR assumes a 5′ location with respect to the transforming genes or *tk*. However, if this is the only mechanism of stimulation of *v-mos* and *tk*, then it might be expected that similar results would be obtained with both genes. The difference in enhancement of *tk* and *v-mos* transforming ability by unlinked LTRs may in part be due to differences in the molar ratios of the DNAs and slightly different transfection procedures. Alternatively, specific sequences present in *v-mos* or the Mo-MSV LTR and not found in the $SFFV_P502$ LTR or *tk* fragments may allow for alternative mechanisms of synergistic enhancement of expression of *v-mos* by the Mo-MSV LTR.

The observation that a retrovirus LTR can activate a silent gene is consistent with recent observations on the mechanisms of viral carcinogenesis by ALV (13–16). These studies have suggested that induction of bursal tumors by ALV involves activation of specific cellular sequences by the insertion of the ALV

LTR adjacent to these sequences (16). A corollary of this promoter insertion model for viral carcinogenesis is that integration of an LTR adjacent to any cellular coding sequence may activate expression of these sequences. The observations presented in this paper provide direct evidence that retrovirus LTRs can indeed activate heterologous genes.

There are several implications of the present results for the analysis of animal cell genetics. First, the frequency that recipient cells express transfecting DNA sequences decreases with time until a stable state is formed (37, 38), because this donor DNA is either rapidly lost or assumes a transcriptionally inactive confirmation. Covalent ligation of a retrovirus LTR to donor DNA sequences may enhance expression by facilitating both the stable maintenance of these DNA sequences, as suggested by Blair *et al.* (34), and their subsequent expression into mRNA transcripts in heterologous host cells. Second, the mammalian genome may contain genes whose expression cannot be detected when the genes are transferred into those cell types currently useful for transfection experiments. Such genes might include those under developmental control, or those, like *c-mos*, that are apparently not expressed, or expressed at very low levels, in normal cells. Association of such cellular sequences with a retrovirus LTR may activate their expression and hence permit their detection, after gene transfer. Third, the results presented in this study, together with similar observations on LTR activation of *onc* sequences (13, 16, 34–36) suggest that there is a strong functional parallel between retrovirus LTRs and bacterial transposable elements in that the insertion of either of these elements into the host genome can lead to activation of expression of adjacent genes. The presence of transposable elements in the genome of bacteria also results in a number of other genetic events, including inactivation of genes, deletions and inversions of sequences adjacent to these elements, and transposition to new sites in the genome (39). It will be of interest to determine whether LTRs, or LTR-like elements, can also mediate similar genetic events in animal cells.

Note Added in Proof. Lee *et al.* (40) have recently demonstrated that the mouse mammary tumor virus LTR can activate expression and glucocorticoid responsiveness of heterologous coding sequences.

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