The long terminal repeat region of the mouse mammary tumour virus contains multiple regulatory elements

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ABSTRACT

Mouse mammary tumour virus (MMTV) is the major aetiologic agent of mouse mammary tumour formation. The expression of this virus is regulated by steroid hormones and cell type specific factors. The nucleotide sequence that controls the steroid hormone response has already been localized between −202 and −59 upstream of the start of transcription in the long terminal repeat (LTR) region of the proviral DNA. Through transfection experiments in three different cultured mouse cell lines (NIH3T3, NMuMG and GR), we have investigated which sequences in the MMTV LTR play a role in the cell type specific expression at the proviral promoter. We have identified two elements on the MMTV LTR from −631 to −560 and from −428 to −364 that have the potential to influence expression at the MMTV LTR promoter. The −631 to −560 element mediated a negative response in all the cell types we studied whereas the −428 to −364 element had negative effects in the mouse fibroblast NIH3T3 and the normal mouse mammary gland cell NMuMG but not in the mouse mammary tumour epithelial GR cells. The −428 to −364 element therefore contributes to the cell type specific expression at MMTV LTR promoter. We have also identified another regulatory element between −1094 and −739 that had a slight positive regulatory effect at the MMTV LTR promoter but greatly enhanced expression at a foreign promoter when present at this promoter in an orientation that is the reverse of its own orientation in the MMTV LTR. This orientation-dependent effect was only observed in the mouse mammary epithelial cells NMuMG and GR but not in mouse fibroblastic cell line NIH3T3. This element may be important in regulating the expression of neighbouring genes in a cell type specific manner. These results show that the MMTV LTR contains multiple regulatory elements necessary for the control of expression at its own promoter and the expression of neighbouring genes.

INTRODUCTION

Mouse mammary tumour virus (MMTV) is a latently oncogenic retrovirus whose increased expression is linked with the development of mammary cancers in mice (for review see refs 18 and 22).

This virus is transmitted through milk during feeding of new born offsprings but mammary tumours rarely arise in the infected animals before 4 months of age (18). The virus remains in infected mammary epithelial cells as a proviral copy and its expression is subject to the effects of various factors that control differentiation and proliferation of the mammary epithelium (9). Most female mice express elevated levels of MMTV during pregnancy and shed high levels of this virus in their milk after parturition (18). The high level of MMTV expression during pregnancy has been shown to be due to pregnancy dependent factors, notably the steroid hormone progesterone (5,9). Progestins induce the expression of MMTV through DNA sequences on the long terminal repeat (LTR) region of the proviral DNA termed the hormone response element (HRE) located between −202 and −59 upstream of the start of transcription at the MMTV LTR promoter (5,6). In addition to the hormone induced expression of MMTV, an elevated level of expression of this virus is found in mouse mammary adenocarcinoma cells but not in normal mammary gland cells (18) nor cells derived from sarcomatous transformation of the mammary epithelium (37, 38). These indicate that certain cell specific factors may also control MMTV expression. These factors or the sequences that mediate their effects have not yet been identified.

The role of MMTV in mouse mammary tumour formation is the activation of a number of proto-oncogenes (int-genes) through the process of insertional mutagenesis. At least four unrelated genes (int 1 to 4) serve as targets for insertional mutagenesis during MMTV induced carcinogenesis (11,12,25,27,31). MMTV insertions in isolated tumours usually reside outside the int-coding sequence either upstream of the gene in the opposite transcriptional orientation or downstream in the same orientation. These findings imply that orientation specific enhancer elements are located on the MMTV proviral DNA that are required for the activation of the int-genes. These elements have also not been identified.

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In this paper, we describe transfection experiments that have helped identify different regulatory elements on the MMTV LTR. One of the elements showed a cell-type specific response and may contribute to the differential MMTV expression in mammary tumour and non-tumour cells. Another element mediated a high level of expression at a heterologous promoter in a cell type- and orientation-specific manner reminiscent of the activity of the element on the MMTV proviral DNA postulated to enhance the expression of the int-genes. Thus DNA sequences that contribute to the cell type specific expression of the MMTV and neighbouring genes are located on the MMTV LTR.

METHODS AND MATERIALS

Plasmid construction

5'Deletion mutants of the MMTV LTR cloned in front of the CAT gene

The clone pMMTV CAT (5) that contains MMTV LTR sequences −631 to +125 cloned in front of the CAT gene was linearized with Hind III and 5' mutants were generated by Bal 31 digestion. The ends of the plasmids were filled-in by DNA polymerase I and tagged with Hind III linkers. The deletion mutants were digested with Hind III/Sac I and the resulting MMTV LTR fragments were isolated and cloned in place of the Hind III/Sac I MMTV LTR fragment of pMMTV CAT (5).

Plasmid pLTR CAT 9 (−1232/+125) was cloned by substituting the 529 bp MMTV LTR sequence from the Bam HI/Sac I (−631/−102) fragment of construct pMMTV CAT (5) with the 1130 bp LTR Bgl II/Sac I (−1232/−102) fragment of pl 2.6 (19). Constructs pHCwt and pGRE00CAT have already been described (8).

MMTV LTR-TK-CAT constructs

Plasmid pTK CAT 7A was constructed by isolating the Hind III/Sau 3A (−631/−449) fragment of pMMTV CAT (5) and cloning it into the Hind III/Bam HI sites of the plasmid pTK CAT 3 (5). Constructs pTKCAT 15 and 12 were similarly cloned by isolating the Hind III/Sau 3A (−581/−449 and −531/−449) fragments of the 5'deletion mutants pHC 15 and 12 and cloning them as described for pTK CAT 7A in front of the TK promoter of pTK CAT 3.

The construct pTKCAT 60 was cloned by isolating the MMTV LTR fragment Bam HI/Ava II (−631/−560) from pMMTV CAT tagging their ends with Bam HI linkers after they have been blunted by DNA polymerase I, and cloning them in the direct or indirect orientations in front of the TK promoter of plasmid pBLCAT 8^+ (20). The orientations of the inserted fragments were determined by DNA sequencing. Construct pTK CAT 5A has been previously described (5). Construct pTK CAT 9A was obtained by cloning the MMTV LTR Hind III/Rsa I (−428/−364) fragment in the direct orientation in the Sal I site of pTKCAT3 (5) by blunt end ligation. Thereafter a Hind III/BamHI fragment containing the insert was cloned into the HindIII/BamHI sites of the vector pBL-CAT-9^+ (20) allowing the MMTV-LTR fragment to be now in the reverse orientation to the direction of transcription of the TK promoter.

Plasmid pTK CAT E1 was constructed by isolating the MMTV LTR fragment −1094 to −739 (Hha I/Rsa I) from pLTRCAT 9 and cloning it in the direct orientation into the Sal I site in front of the TK promoter of pBL-CAT-8^+ after the Sal I site had been blunted by DNA polymerase I. Thereafter a HindIII/BamHI fragment was isolated and cloned in the corresponding sites in vector pBL-CAT-9^+ as described above. The orientations of the inserts were determined by DNA sequencing. In all the constructs the denotation syn represents the orientation as the fragment occurs in the MMTV LTR and anti refers to the reverse orientation.

Cell culture and transfection

Normal mouse mammary gland cell NMuMG (29), mouse mammary tumour cell line GR (35) and mouse fibroblastic cell line NIH3T3 were cultured in Dulbecco's modified Eagles medium (DMEM) supplemented with 10% fetal calf serum (FCS). Transient transfection into $5-8\times10^5$ NMuMG or GR cells were carried out with 10 μg plasmid DNA and where indicated with an additional 2 μg of pCH110 (Pharmacia) using the calcium phosphate method of Ott et al., 1984 (28). The construct pCH110 contains the SV40 promoter driving the

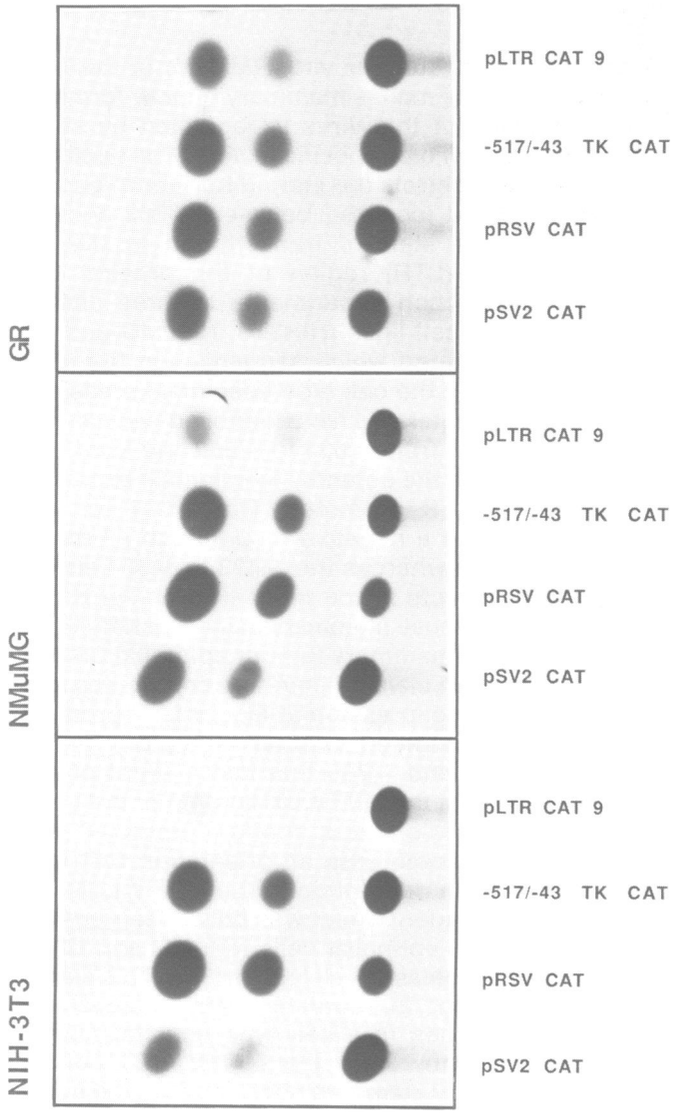

Fig.1 *CAT activity in various mouse cell lines transfected with pLTRCAT9 and three control plasmids.*
The MMTV LTR-CAT construct, pLTRCAT9 and three control plasmids, pSV_2CAT, pRSVCAT and −517/−43 TKCAT were transiently transfected into the indicated cells. Extracts from these cells containing equal amounts of protein were used for CAT assay as described by Gorman et al., 1982 (14). The CAT activity mediated by the various constructs in the different cells are indicated.

transcription of the β-galactosidase gene, used for standardizing the transfection experiments.

CAT assay

Forty hours after transfection, the cells were disrupted by repeated freezing in dry ice-ethanol bath and thawing at 37°C (five times). Cellular extracts containing 200 μg protein or identical amounts of β-galactosidase were used for CAT assay according to the procedure described by Gorman et al., 1982 (14).

Table 1. *The expression of the MMTV-LTR-CAT construct pLTR CAT 9 in various mouse cell lines in relation to the expression of three control plasmids*
The MMTV LTR-CAT construct, pLTR CAT9 and three control plasmids pSV_2CAT, pRSVCAT and −517/−43 TK CAT were transiently transfected in parallel into NIH3T3, GR and NMuMG cells and CAT activity was determined in extracts of the transfected cells. CAT activity was determined as % acetylated chloramphenicol per mg protein. The results are presented as the ratio of the CAT activity of pLTR CAT 9 to the CAT activity of the control plasmids. These results are the mean ± standard deviation of three independent experiments.

	Relative CAT Activity of pLTR CAT 9		
Control Plasmids	GR cells	NMuMG cells	NIH-3T3 cells
pSV_2 CAT	0.28 +/-0.04	0.11 +/-0.02	0.10 +/-0.02
pRSV CAT	0.33 +/-0.04	0.08 +/-0.01	0.04 +/-0.02
-517/-43 TK CAT	0.27 +/-0.02	0.09 +/-0.01	0.05 +/-0.01

β-galactosidase assay

Extracts from the cells cotransfected with MMTV LTR chimaeric constructs and pCH110 were used for the determination of β-galactosidase activity as described by Herbomel et al., 1984 (16). Extracts with identical amounts of β-galactosidase were then used for determination of CAT activity.

RESULTS

MMTV LTR chimaeric constructs are differentially expressed in various cultured mouse cells

The level of expression at the MMTV LTR promoter was shown to be different in three cultured mouse cell lines in transient experiments with a chimaeric construct, pLTR CAT 9, consisting of the entire MMTV LTR cloned in front of the bacterial chloramphenicol acetyl transferase (CAT) gene (Fig. 1, Table 1). The cell lines used for the transfection were the mouse fibroblastic cell line NIH3T3, the normal mouse mammary gland cell line, NMuMG (29) and the mouse mammary tumour cell line, GR (35). To correct for differences in transfection efficiencies, three control plasmids that do not show large differences in their expression in the cell lines tested were transfected in parallel with the pLTR CAT 9 construct. The

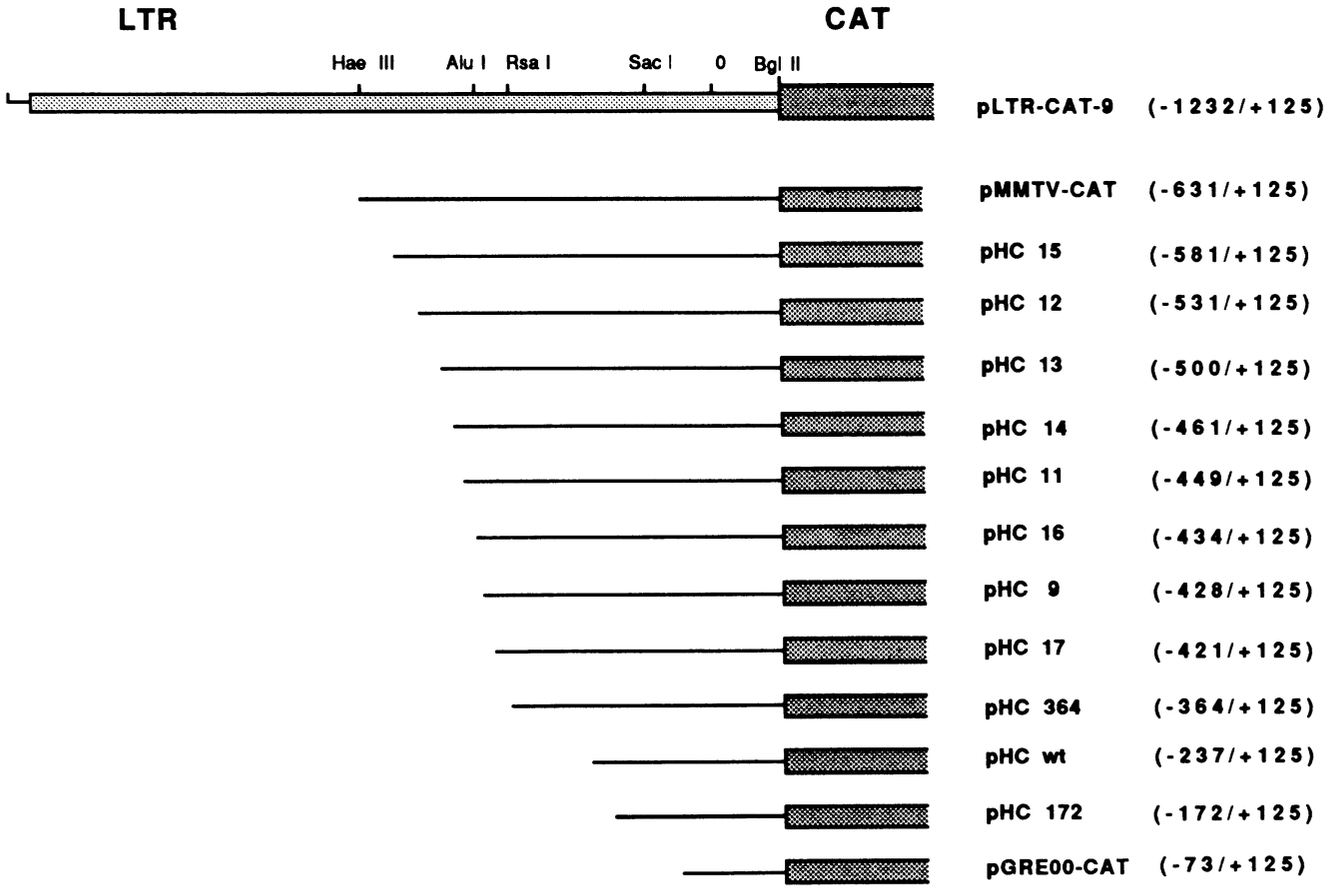

Fig. 2 *Schematic representation of the various 5'deletion mutations in the MMTV LTR*
The indicated 5'deletion fragments of the MMTV LTR were all cloned in front of the CAT gene in such a way that the MMTV LTR promoter controls transcription of the CAT gene.

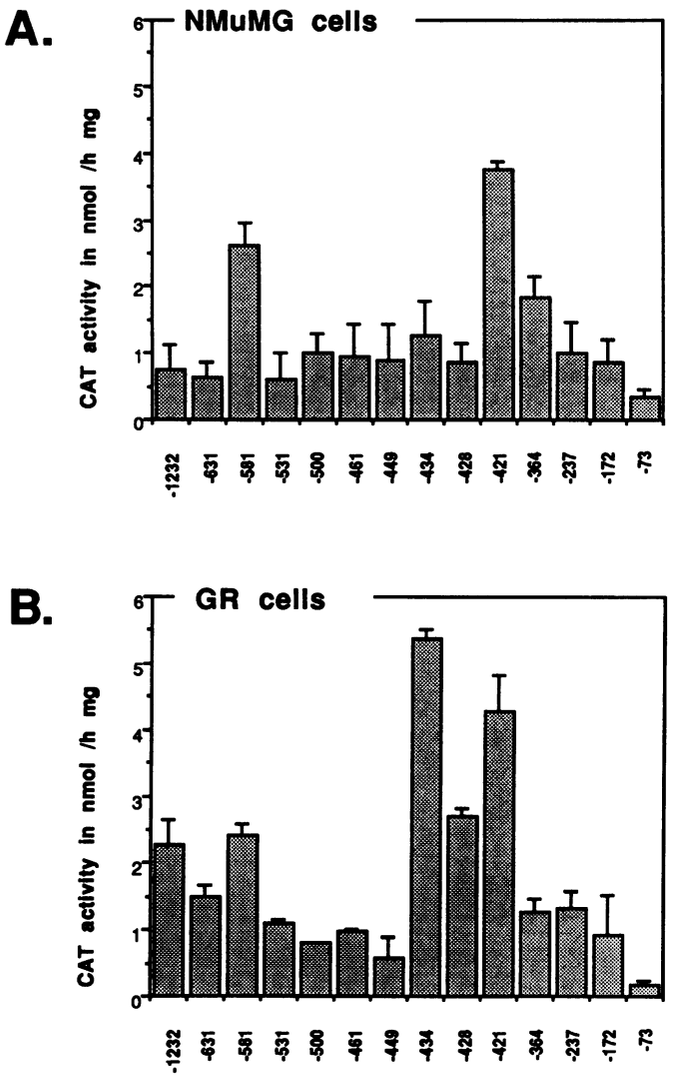

Fig.3 *Expression of chimaeric constructs containing various 5'deletion mutations in the MMTV LTR in two different mouse mammary epithelial cells*
The MMTV LTR CAT constructs containing various 5'deletion mutations in the MMTV LTR were transiently transfected into the mouse mammary epithelial cells NMuMG and GR followed by the determination of CAT activity 40 h later. The bar diagram represents the mean ± standard deviation of at least 3 different experiments. The limits of the mutations at the 5'end of the various mutations are indicated.

promoter activity of the MMTV LTR was expressed with reference to the control plasmids. The 3 plasmids used were pSV_2CAT (14), and pRSV CAT (13), in which the SV40 and RSV promoters drive the transcription of the CAT gene as well as −517/−43 TK CAT in which the indicated sequence from the human collagenase gene controls transcription of the thymidine kinase (TK) promoter linked to the CAT gene (1). The CAT activity of pLTR CAT 9 in relation to the activity of the 3 controls showed in each case a higher level of expression of the MMTV LTR construct in the mammary tumour GR cells than in the non-tumorigenic mammary NMuMG cell line or the fibroblastic cell line NIH3T3 (Fig.1, Table 1). A quantitation of the relative activity of the MMTV LTR promoter in these cell lines showed a 3−8 fold higher level of expression in the tumour cells than in the non-tumour cells (Table 1). This indicates that GR cells contain factors that enhance MMTV expression through sequences located on the MMTV LTR.

The expression of chimaeric constructs containing different 5'deletion fragments of the MMTV LTR in NMuMG or GR cells show multiple regulatory elements in the MMTV LTR

To determine the regions of the MMTV LTR responsible for the higher level of expression at the MMTV LTR promoter in the GR, we compared the expression pattern of various 5'deletion fragments of the MMTV LTR after transfection into GR and NMuMG cells. All the transfected constructs contained the MMTV LTR promoter driving transcription of the CAT gene (Fig.2). Analyses of CAT activity in the transfected cells revealed a complex pattern of CAT activity possibly generated by an interplay of positive and negative regulatory elements (Fig. 3). The results further indicated vaguely the presence of two regulatory element at −430 and −600 upstream of the MMTV LTR promoter that may differentially affect expression at the MMTV LTR promoter in the cell lines studied (Fig.3 compare A and B).

MMTV LTR sequences between −631 to −560 mediate a negative expression at a heterologous TK promoter

As the functional activity of different sequences identified in the 5'deletion analyses could be masked by the contribution of their neighbouring 3' sequences and thus complicate the results, we isolated fragments from the two main regulatory regions we have identified in the MMTV LTR and determined their ability to regulate the expression at a foreign promoter. The various MMTV LTR fragments were cloned in front of the herpes simplex TK promoter linked to the CAT gene (Fig.4) and the constructs were transfected into the various mouse cell lines. The ability of the MMTV LTR fragments to regulate expression at the TK promoter positively or negatively, is measured from the ratio of the promoter activity of the different MMTV LTR-TK CAT constructs to the activity of the TK-CAT construct without an insert.

The MMTV LTR sequence from −631 to −449 in pTK CAT 7A (Fig.4) had a negative effect on the expression at the TK promoter in all the cell lines tested (Table 2). Deletion at the 5'end of this sequence generated fragments −581 to −449 and −531 to −449 which no longer mediated a negative response but instead had a slight positive influence at the TK promoter (pTK-CAT-15 and pTK-CAT-12; Table 2). This indicates that the negative regulatory element is situated at the 5'-end of the −631 to −449 sequence. A fragment −631 to −560 isolated from the 5'-end of the −631 to −449 sequence indeed mediated a negative response at the TK promoter (pTK-CAT-60; Table 2).

The negative regulatory activity of the −631 to −560 fragment was only observed when the fragment was cloned in front of the TK promoter in the same orientation as in the MMTV LTR promoter (pTK CAT 60 syn; Table 2). In the reverse orientation this fragment had no significant effect on expression at the TK promoter in all three cell lines tested (pTK CAT 60 anti, Table 2). Thus the sequence −630 to −560 in the MMTV LTR context must have a negative regulatory effect on expression only at the MMTV LTR promoter and not on other promoters upstream of the MMTV LTR.

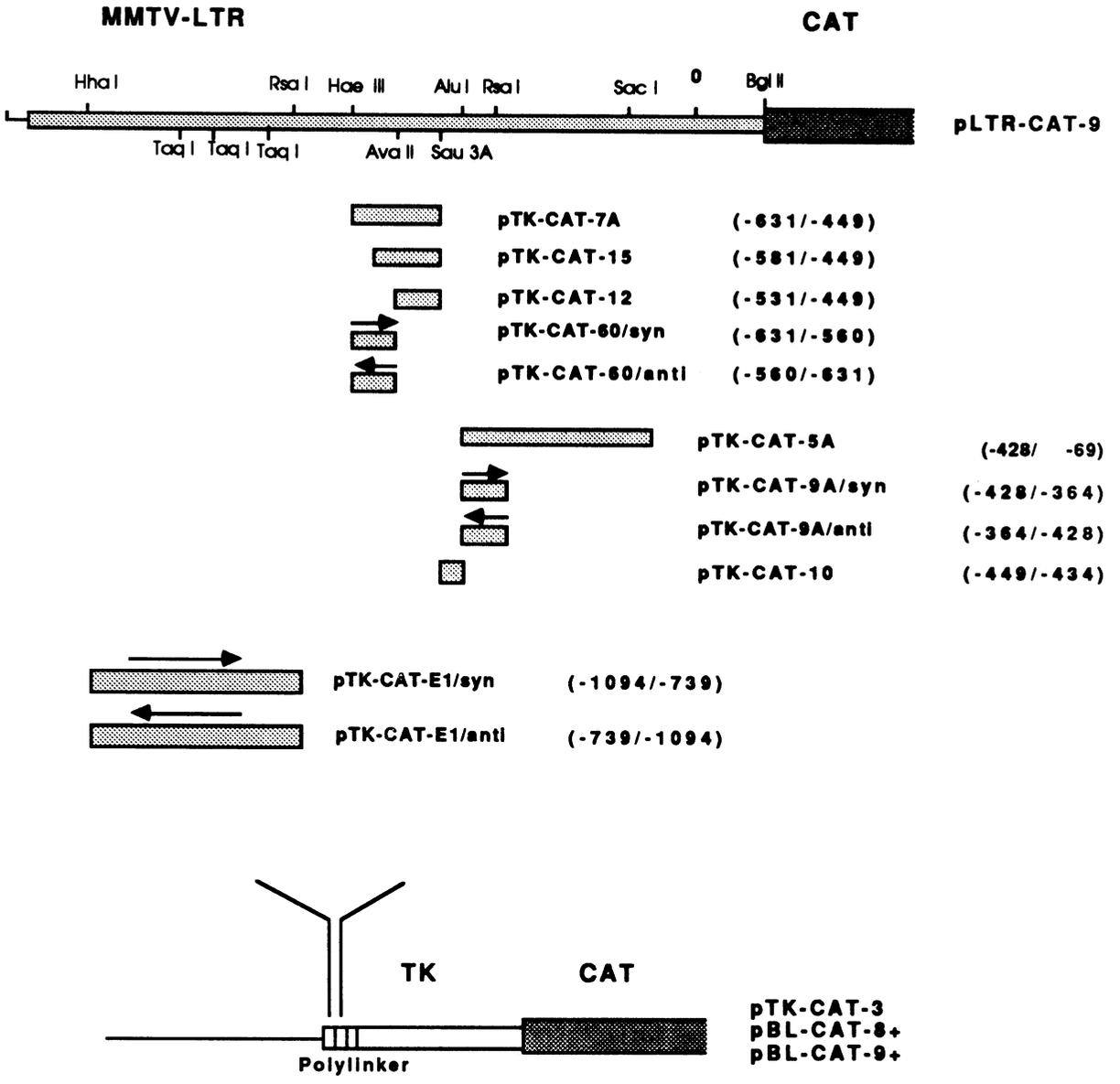

Fig.4 *Schematic representation of the various MMTV LTR fragments cloned in front of TK-CAT constructs*
The indicated MMTV LTR sequences were isolated and cloned in the polylinker region in front of the TK promoter coupled to the CAT gene. To ensure easy cloning of these fragments, various TK CAT constructs were used. These TK-CAT constructs, pTK CAT 3 (5) and pBL CAT 8^+ or pBL CAT 9^+ (20) all contained the TK promoter (−105/+52) linked to the CAT gene but with different polylinker region at −105 of the TK sequence. Constructs with the denotation (syn) refer to those in which the MMTV LTR fragment is in the same orientation as it occurs in the MMTV LTR, whilst (anti) represents the reverse orientation.

MMTV LTR sequences localized between −428 and −364 mediate a cell type specific expression at a heterologous TK promoter

Sequences within the MMTV LTR from −428 to −69 when placed in front of the TK promoter (pTK-CAT-5A, Fig.4) mediated a cell type specific effect in transfection experiments. This fragment repressed expression at the TK promoter in NIH3T3 and NMuMG cells to 63% and 58% respectively but slightly enhanced expression at this promoter in the GR cells. This cell type specific effect of the −428 to −69 fragment can be narrowed down to a 64 bp fragment located between −428 to −364 (Table 2, pTK CAT 9A). The −428 to −364 sequence, unlike the −631 to −560 negative regulatory element, influences expression in two different orientations in front of the TK promoter (Table 2, pTK CAT 9A syn and anti). Its activity can therefore be manifested not only at the MMTV LTR promoter in the proviral situation but also on genes that may lie away from the direction of expression of the MMTV LTR promoter. A 15 bp element that corresponds to MMTV LTR sequences −449 to −434 synthesized chemically with the Pharmacia gene machine and cloned in front of the TK promoter (Fig.4, pTKCAT 10) had only a slight negative influence on expression at the TK promoter in NIH3T3 and NMuMG cells (Table 2). This indicates that the −449 to −434 sequence alone is not very important although it can still in conjunction with the sequence at −428 to −364 mediate a cell-type response at the MMTV LTR promoter.

Table 2. *Effect of different MMTV LTR fragments on expression at the TK promoter*
The indicated MMTV LTR fragments were cloned in front of the TK CAT constructs indicated in Fig.4. Chimaeric constructs with the denotation (syn) are those in which the MMTV LTR fragment is cloned in the orientation it occurs in the MMTV LTR and (anti), the reverse orientation. The chimaeric constructs were co-transfected with the β-galactosidase construct pCH 110 (16) for standardization of transfection efficiency. CAT assay was performed with cellular extracts containing the same β-galactosidase activity and the results were presented as the ratio of CAT activity of the MMTV-TK-CAT constructs to the CAT activity of the TK-CAT construct without insert. The above results are the mean ± standard deviation of 5 to 7 independent experiments with at least 2 different plasmid preparation.

		Relative CAT Activity		
Constructs	MMTV-Fragment	GR cells	NMuMG cells	NIH-3T3 cells
pTK-CAT-7A	(-631-449)	0.28 +/-0.09	0.16 +/-0.07	0.18 +/-0.03
pTK-CAT-15	(-581/-449)	1.44 +/-0.04	1.34 +/-0.08	1.35 +/-0.12
pTK-CAT-12	(-531/-449)	1.45 +/-0.17	1.41 +/-0.07	1.38 +/-0.24
pTK-CAT-60syn	(-631/-560)	0.45 +/-0.02	0.42 +/-0.02	0.49 +/-0.09
pTK-CAT-60anti	(-560/-631)	1.23 +/-0.07	1.12 +/-0.09	1.08 +/-0.12
pTK-CAT-5A	(-428/- 69)	1.68 +/-0.57	0.58 +/-0.16	0.63 +/-0.21
pTK-CAT-10	(-449/-434)	0.98 +/-0.13	0.78 +/-0.19	0.81 +/-0.23
pTK-CAT-9Asyn	(-428/-364)	1.57 +/-0.11	0.40 +/-0.16	0.14 +/-0.12
pTK-CAT-9Aanti	(-364/-428)	0.97 +/-0.23	0.51 +/-0.11	0.49 +/-0.06
pTK-CAT-E1syn	(-1094/-739)	2.40 +/-0.49	1.46 +/-0.25	1.17 +/-0.31
pTK-CAT-E1anti	(-739/-1094)	7.03 +/-2.34	2.91 +/-0.68	1.44 +/-0.26

MMTV LTR sequences between −1094 to −739 contain element(s) capable of enhancing the expression of neighbouring genes

Deletion of MMTV LTR sequences between −1232 to −631 slightly repressed the activity at the MMTV LTR promoter in both NMuMG and GR cells (Figs. 3A and B) indicating that they contain positive regulatory elements. This effect was more noticeable in GR cells than in NMuMG cells. To study the influence this region has on the activity of TK promoter, we isolated a 355bp fragment (−1094 to −739) by HhaI/RsaI restriction endonuclease digest that allowed for easy cloning and inserted it in front of the TK promoter. The −1094 to −739 construct slightly enhanced the activity of the TK promoter in GR cells (Table 2; pTK-CAT-E1 syn). More important, it showed a clear increase in expression at the TK promoter in GR cells when it was cloned in the reverse orientation to how it occurs in the MMTV LTR (Table 2, pTK-CAT-E1 anti). The increased expression in the reverse orientation at the TK promoter was also observed in NMuMG cells, but not in NIH3T3 cells (Table 2; pTK-CAT-E1 anti). That the −1094 to −739 fragment affects expression at a promoter in an orientation and cell type specific manner, makes it a good candidate for activating genes outside the MMTV proviral sequence only in specific cell types. This orientation-specific enhancing property of the −1094 to −739 fragment is a hallmark of the action of the activator on the MMTV proviral DNA postulated to enhance expression of the int-genes. We therefore conclude that sequences on the MMTV proviral DNA required for activation of the int-genes are most probably located within −1094 and −739 on the MMTV LTR.

DISCUSSION

We have demonstrated in this study that the MMTV LTR contains multiple regulatory sequences. Three different regulatory elements were identified through systematic 5′deletion analyses and a study of the functional activity of specific MMTV LTR fragments cloned in front of the heterologous TK promoter. Two of these elements that extend from −631 to −560 and −428 to −364 may influence expression at the MMTV LTR promoter. A third element localized between −1094 and −739 slightly increases expression at the MMTV LTR promoter and in addition it is potential enhancer of the activity of neighbouring genes.

We have identified the fragment between −631 to −560 upstream of the MMTV LTR transcriptional start site as a sequence with negative regulatory properties on expression at the MMTV LTR promoter. When placed in front of the TK promoter, this element also down regulates expression at this promoter. Previously, we have shown in transfection experiments with MMTV LTR chimeric constructs in the feline kidney cell line CRFK that sequences between −631 to −428 decreased expression at this viral promoter (8). Hsu et al., 1988 (17) have also shown in transfection experiments in the the mink lung cell line, CCL-64 that MMTV LTR sequences between −637 to −225 contain sequences that down-regulate expression at the MMTV LTR promoter. Although in the above two studies the sequences that mediate the negative response were not narrowed down to relatively short nucleotide sequences, it is conceivable that the 71 bp sequence between −631 and −560 that we have identified in this work could have contributed to the down-regulation of MMTV expression in the CRFK and mink lung cell lines. Thus the −631 to −560 MMTV LTR fragment most probably mediates negative control of expression at the MMTV LTR promoter in a large variety of cells.

We have identified another region between −428 and −364 in the MMTV LTR that may have a cell type negative regulatory function at the MMTV LTR promoter. This sequence when cloned in front of the TK promoter represses expression at this promoter in a cell type specific manner. The MMTV LTR regulatory sequence −428 to −364 that we have identified is contained in a larger MMTV LTR fragment (−455 to −364) identified by Morley et al., 1987 (21) as a negative regulatory element in mouse fibroblast. Indeed in the mouse fibroblast NIH-3T3 and in the mouse mammary cell line NMuMG our −428 to −364 MMTV LTR fragment has also negative regulatory properties. The same sequence however does not have a negative regulatory action in GR cells. It has a slight positive effect on expression at the TK promoter in these cells. This fragment may therefore play a role in mediating cell type specific expression at the MMTV LTR promoter.

Unlike the −631 to −560 fragment that negatively influences expression at the MMTV LTR promoter only in one orientation, the −428 to −364 fragment functions in both orientations when cloned in front of the TK promoter as also reported by Morley et al., 1987 (21). This indicates that it has the ability to influence the expression at the MMTV LTR promoter as well as the promoters of neighbouring genes. As the −428 to −364 fragment has negative regulatory properties in some cells but not in all cells and from its proximity to the −631 to −560 general negative regulatory element, it is tempting to postulate that it co-operates with this element for an effective repression of MMTV LTR promoter activity in non-expressing cells. Whether this is the way cell specific expression of MMTV is achieved is yet to be determined. Negative regulatory elements are reportedly present

in the HRE (−202 to −59) of the MMTV LTR too (40). In this case, these sequences have been postulated to control the hormone response at the MMTV LTR promoter.

Negative regulation of gene expression, a common mechanism of gene control in prokaryotes and in yeast (4,34), has only relatively recently been documented in higher eukaryotes. Cis-acting negative regulatory sequences have now been defined for several eukaryotic genes. In the insulin I (24), the α-fetoprotein (23) and the retinol-binding protein (10) genes as well as in several viral enhancers (15), negative regulatory elements prevent expression in non-expressing cells. In expressing cells, a lack of negative regulatory factors to interact with negative regulatory sequences could lead to an increased expression of genes in a cell type specific manner. Such actions of negative regulatory elements in mediating cell type gene expression have been reported in the chicken lysozyme gene (2) and the embryonic skeletal myosin heavy chain gene (3). Whether the increased expression at the MMTV LTR promotor in GR cells is due to the loss of negative regulatory factors in these cells or the presence of other regulatory factors that interfere with the action of the negative factors is not known.

We have also shown that MMTV LTR sequences between −1094 to −739 have regulatory function. This region coincides with MMTV LTR sequences (−1185 to −741) shown by Stewart et al., 1988 to mediate a high level of expression of fusion genes in epithelial cells of a member of organs in transgenic mice (39). Consistent with the report of Stewart et al., 1988 (39) we have shown that our −1094 to −739 fragment has a slight positive effect on expression of the linked TK promoter in the mammary epithelial cells GR and NMuMG. More important, we have shown that the ability of the −1094 to −739 fragment to increase expression at the heterologous TK promoter is more pronounced when this fragment is in the reverse orientation to its own orientation in the MMTV LTR.

Several analyses of MMTV-host junction fragments from mouse mammary tumours have shown that the MMTV provirus is integrated outside the coding region of the int-genes in different tumours across at least 20Kb sequence either upstream of the int-genes in the opposite orientation or downstrem in the same orientation (for review see Ref. 30). At either integration site the −1094 to −739 fragment that we have defined would be in the reverse orientation to the direction of transcription of the int-genes, making it a likely candidate for the int-gene activator sequence.

The enhancing activity of the −1094 to −739 element is cell type specific; it is more active in the mouse mammary tumour cell line GR, than in the normal mouse mammary cell NMuMG or the mouse fibroblastic cell line NIH 3T3. A major role of this fragment in activation of the int-gene would however have to accommodate a high expression of its orientation-dependent function in normal mammary epithelial cells where the int-activation initially occurs. The results presented in this work show that the GR cells differ from the normal mammary cell and the fibroblastic cell lines more than the fibroblast from the mammary cells. This indicates that transformation specific factors in the GR cells may contribute significantly to the activity of the −1094 to −739 fragment. Identification and characterization of other cis- and trans-acting elements that control the function of this fragment may perhaps clarify these points. Future deletion analyses will also reveal whether the 355 bp (−1094 to −739) MMTV LTR uni-directional activator element can be narrowed down to shorter nucleotide sequences or whether like the 143 bp HRE on the MMTV LTR (7,33) it is composed of multiple elements that work in concert to generate a functional unit.

Whatever the *in vivo* function of the −1094 to −739 sequence, we have demonstrated in this work that in addition to the hormone response element, the MMTV LTR contains other regulatory elements that are responsible for cell type specific expression at its promoter and for activation of neighbouring genes. The MMTV LTR therefore contains multiple regulatory elements that are almost undoubtedly utilized by this virus in mammary tumour formation in the mouse.

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