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The MLV and SV40 enhancers have a similar pattern of transcriptional activation

P.Augereau* and B.Wasylyk

Laboratoire de Génétique Moléculaire des Eucaryotes du CNRS, Unité 184 de Biologie Moléculaire et de Génie Génétique de l'INSERM, Institut de Chimie Biologique, Faculté de Médecine, 11, rue Humann, 67085 Strasbourg Cédex, France

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ABSTRACT

Activation of transcription by the Moloney Murine Leukemia Virus (MLV) and Simian Virus (SV40) enhancers was compared by transfecting recombinants containing these enhancers in either mouse or human cell-lines, and analysing RNA 48 h later by quantitative S1 nuclease mapping. The enhancers share the following properties. They stimulate transcription in an orientation-independent manner from the same startsites on the natural heterologous conalbumin (+62 to -102) or SV40 early promoter elements as well as on substitute promoter elements. The enhancers are most efficient when they are located directly upstream from the conalbumin (+62 to -102) promoter element, but they still stimulate transcription when they are either immediately downstream from the promoter element, or further upstream. Increasing the distance by interposing DNA sequences between the enhancers and the conalbumin promoter fragment results in decreased activation. Both enhancers show some cell-line specificity for activation of transcription. However, in all cell-lines and constructions tested the MLV enhancer was always less efficient than the SV40 enhancer. These results suggest that the MLV and SV40 enhancers stimulate transcription by similar mechanisms.

INTRODUCTION

Enhancers are characterised by their ability to stimulate transcription in an orientation-independent manner from homologous, heterologous and substitute promoter elements over considerable distance (several thousand bp) (for reviews see 1-4). One of the most thoroughly characterised enhancer is the SV40 72 bp repeat (for refs. see 5-11). In particular we have shown that proximal potential promoter sequences are activated in preference to more distal ones (the "distance" effect, 12, 13). These results are consistent with the entry site model of Moreau et al. (7) which proposes that some component of the transcription machinery "enters" the DNA at the enhancer, and then scans the DNA for promoter elements in such a way that promoter elements which are close to the enhancer are stimulated in preference to more distal elements.

To see whether other enhancers operate through the same mechanism we

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have compared directly, in similar constructions, the SV40 enhancer with the enhancer from the retrovirus Moloney murine leukemia virus (MLV) (Fig. 1). The proviral DNA is bounded by long terminal repeats (LTR's) (for reviews and refs. see 14-16), each of which contains transcriptional control sequences including typical promoter elements (17, 18) and an enhancer (19-22). Recombinants containing either the MLV or SV40 enhancers were transfected in several cell-lines and the RNA was analyzed by quantitative S1 nuclease mapping. We find that the MLV enhancer has many of the properties of the SV40 enhancer. It stimulates transcription, in an orientation independent manner, from two heterologous promoter elements [conalbumin (+62 to -102) and SV40 early] and from the same substitute startsites (startsites with no-known natural counterpart) as the SV40 enhancer. Stimulation is observed when the enhancer is upstream or downstream from the promoter, and it decreases with increasing distance of the enhancer from the promoter element. However, the MLV enhancer has a different cell-line specificity, and is less efficient than the SV40 enhancer. These results suggest that the SV40 and MLV enhancers stimulate transcription by similar mechanisms, and that the entry-site model may apply to both enhancers.

MATERIALS AND METHODS

Construction of recombinants :

The recombinants pTCT, pTCTB, pTCTB3 and pTBCT have already been described (7). A similar nomenclature for the recombinants has been used in this study. The letters L, S and B represent the MLV, MSV and SV40 enhancers, respectively.

A DNA polymerase I repaired Sau 3A (-353) to XbaI (-151) fragment (see Fig. 1) from pMLV-1A (23) was ligated to BamHI linkers, digested with BamHI and ligated to pTCT which had been partially digested with BamHI. Recombinants with the MLV enhancer fragment in either orientations and either upstream (pTCTL and pTCTLI) or downstream (pTLCT and pTLICT) of the conalbumin promoter fragment (-102 to +62) were isolated (see Fig. 1). pTCTS has the homologous Sau3A-XbaI fragment from MSV (nucleotides 327 to 529 - a gift from P. Gruss, 24) inserted with BamHI linkers at the same position as the MLV enhancer in pTCTL. The MLV enhancer fragment from pTCTL was repaired with DNA polymerase I and blunt-end ligated to pTCT that had been digested with SalI (650 in pBR322) and repaired with DNA polymerase I (to give pTCTL3 and pTCTLI3, Fig. 1) or to pTCT (tet^+) which had been

digested with PvuI (3737 in pBR322) and repaired with T4 DNA polymerase (25) (to give pTCTL37 and pTCTLI37, Fig. 1).

A modified SV40 early promoter containing fragment extending from HindIII (coordinate 5171, BBB system, 26) to EcoRI (created with a linker at the HpaII site, coordinate 346) was inserted between the HindIII and the EcoRI sites of a pBR322 derivative lacking the BamHI site (a gift from R. Everett). The SV40 fragment was modified from wild type by an in vitro mutation (5'-TAGTCC-3' to 5'-GGATCC-3', nucleotides 106-101) which creates a BamHI site at the 72 bp-21 bp repeat junction (M. Zenke, T. Grunström, H. Matthès and M. Wintzerith, in preparation). A BamHI linker was inserted in the EcoRI site, and the SV40 72 bp repeat and upstream sequences (nucleotides 104-346) were deleted by BamHI cutting. We then inserted, by sticky-end ligation in the BamHI site (upstream from the SV40 early promoter element) either the HS102 fragment A (as a Sau 3A fragment from pTCTB, see Ref. 7) or the MLV and MSV enhancer fragments (as BamHI fragments from pTCTL and pTCTS, see above). The DNA polymerase I repaired HindIII-EcoRI fragments from the resulting recombinants (containing the SV40 early promoter region) were then blunt end ligated to the DNA polymerase I repaired XhoI site of pDB2 (27) to give pSV (no enhancer), pSVB (with the HS102 fragment A), pSVL and pSVLI (with the MLV enhancer in either orientation) and pSVS1 (with the MSV enhancer). pSVS2 is similar to pSVS1 except that the wild type SV40 early promoter was used (i.e. without a BamHI site at the 21 bp-72 bp repeat junction) and that the MSV enhancer (with BamHI linkers at its extremities, digested with BamHI and repaired with Klenow DNA polymerase I) was blunt end ligated between the T4 DNA polymerase repaired EcoRI (1 in pBR322) and SphI (128) sites (see Fig. 1).

Transfection, RNA extraction and S1 nuclease mapping :

Recombinant DNAs were transfected in either NIH3T3, LMTK$^-$ or HeLa cells by the calcium phosphate method of Graham and Van der Eb (28) as modified by Corsaro and Pearson (29), using 15 μg of test DNA and 15 μg of the internal control pβ(244+)β (30) per 75 cm^2 Falcon flask. After 24 h the cells were washed with serum free medium and then incubated for an additional 24 h. Total RNA (31) was analyzed by S1 nuclease mapping in excess probe (32). RNA was quantitated by scanning autoradiogramms and corrected for transcription from the internal control. In certain experiments, duplicate samples of the same RNA were analyzed separately for transcription from the reference plasmid and the test plasmid. At least two

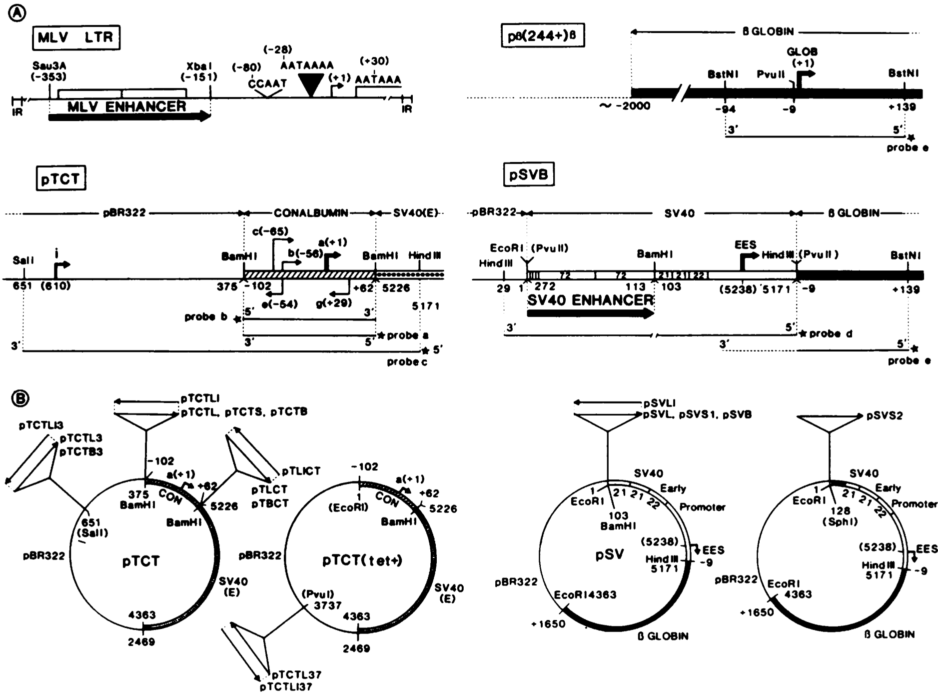

Figure 1 : Structure of the MLV LTR and of recombinants containing the MLV, MSV or SV40 enhancers and conalbumin or SV40-early promoter elements.
A) The MLV LTR and line drawings of pTCT, pβ(244+)β and pSVB. The main features of the MLV LTR are indicated. IR indicates the inverted repeats which bound the LTR. The positions of characteristic sequences and restriction sites are given relative to the capsite (+1). The sequences AATAAAA found downstream (+30) and upstream (-28) from the capsite are polyadenylation and TATA box sequences respectively, and CCAAT is a "CAT box" like sequence (23). The 75 bp repeated sequences are indicated by open boxes. The MLV enhancer fragment [XbaI (-151) Sau3A (-353)] is indicated by a large arrow. The structures of the recombinants are shown in part B, whilst some details of their structure, the position of natural and substitute startsites, and the DNA probes used for S1 nuclease mapping are indicated on the line drawings. The coordinates of startsites and restriction sites lost during cloning are given in parentheses. Where more than one nucleotide is used for RNA initiation, the coordinate indicated corresponds to the most upstream of the prominent starts (for details see 12, 33). pTCT : a, natural conalbumin startsite, b, c, e, g, i : substitute startsites detected with recombinants derived from pTCT by inserting the SV40 and MLV enhancers at various positions ; probes a and b : [^{32}P] 5'-end labelled coding and non-coding strands respectively of the conalbumin (+62 to -102) BamHI fragment ; probe c : [^{32}P] 5'-end labelled coding strand extending from HindIII (5171 in SV40) to SalI (651 in pBR322) of pTCT. pβ(244+)β : recombinant from de Villiers and Schaffner (30) containing two rabbit β globin gene fragments and the polyoma enhancer ; probe e : [^{32}P] 5'-end labelled coding strand of the rabbit β globin BstNI fragment (+139 to -94) ; GLOB : natural startsite of the rabbit β globin gene. pSVB : see

part B. 72, 21, 22 in the open line indicate the 72 bp , 21 bp and 22 bp repeated sequences of the SV40 origin region ; EES : the major early-early RNA startsites of the SV40 early promoter ; SV40 enhancer : the pHS102 fragment A (7) ; probe d : [^{32}P] 5'-end labelled probe extending from HindIII (5171 in SV40) to HindIII (29 in pBR322) from CW12 (33). There is a 10 nucleotide sequence discontinuity between the probe and pSVB (nucleotides 103 to 113 indicated by the break in the line). B) Structure of recombinants containing either the chicken conalbumin or SV40 early promoter elements. The coordinates for CON (the conalbumin gene fragment from +62 to -102), SV40 (E) (SV40 early T-antigen coding region from 2469 to 5226) and β globin (the rabbit β globin PvuII fragment from -9 to about +1650) are given outside the circle, and for the SV40 early promoter element, and pBR322 inside the circle. The arrows outside the circles correspond to enhancer fragments from either MLV (recombinants with L in their name, e.g. pTCTL), SV40 (recombinants with a B, e.g. pTCTB) or MSV (recombinants with an S, e.g. pSVS1). For the MLV and SV40 enhancer fragments see A. The MSV enhancer fragment extends from Sau3A (529) to XbaI (327) of the MSV LTR (24). The directions of the arrows indicate the orientation of the fragment (see A). For other symbols see A.

different DNA preparations of each recombinant was used in different transfections.

RESULTS

A. A DNA fragment containing the 75 bp repeated sequences of the MLV LTR stimulates transcription from heterologous promoter elements.

To demonstrate that the MLV LTR XbaI (-151)-Sau3A (-353) DNA fragment stimulates transcription from heterologous promoter elements, we constructed recombinants containing this sequence in either orientation directly upstream from either the conalbumin (+62 to -102, pTCTL and pTCTLI, Fig. 1) or SV40 early (5171 to 103, pSVL and pSVLI, Fig. 1) promoter regions. These and similar recombinants with the SV40 enhancer (pTCTB, pSVB, Fig. 1) were cotransfected with a reference plasmid pβ(244+)β (containing the rabbit β globin gene and the polyoma enhancer, Ref. 30) in either NIH 3T3 or LMTK$^-$ mouse cells. Total RNA was extracted 48 h later and analyzed by quantitative S1 nuclease mapping using the corresponding 5'-end labelled coding strand probes (probes a and d for conalbumin and SV40 early promoter containing recombinants, respectively, and probe e for pβ(244+)β see Fig. 1). The MLV enhancer, in the direct orientation (i.e. the same orientation relative to transcription from the natural startsite as in the LTR), stimulates transcription from the "natural" conalbumin startsite a approximately 20-fold (compare pTCT and pTCTL in Fig. 2A, lanes 1 and 2, and Fig. 2B, lanes 1, 2, 5 and 6 and Table 1; transcription from startsite a for pTCT was clearly seen on longer exposition of the autoradiograms). Similarly,

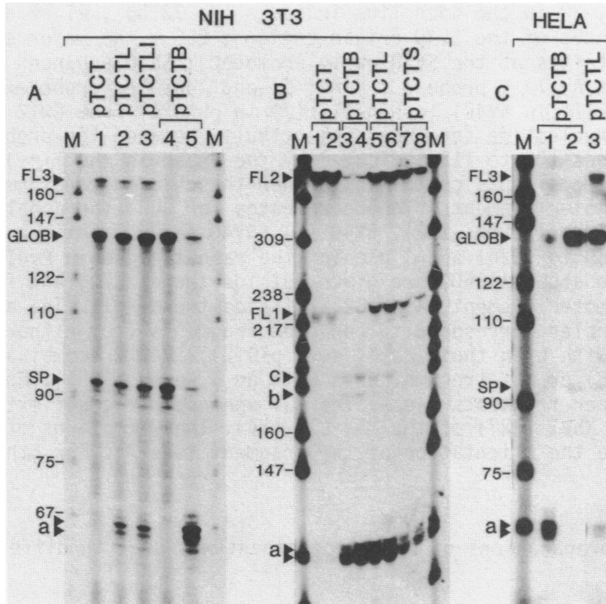

Figure 2 : S1 nuclease analysis of RNA from transfected NIH 3T3 and HeLa cells.
RNA from transfections with pTCT, pTCTB, pTCTL, pTCTLI and pTCTS in NIH 3T3 cells (A and B) or pTCTB and pTCTL in HeLa cells (C) was analysed by S1 nuclease mapping using either a mixture of probes a and e (A, lanes 1-3 and 5 ; C, lanes 1 and 3), probe e alone (A, lane 4 ; C, lane 2), or probe c (B) (see Fig. 1 for probes). The amounts of RNA used were 40 μg (A, lanes 1-4), 4 μg (A, lane 5), 10 μg (B, lanes 1, 2, 5-8), 1 μg (B, lanes 3, 4), 50 μg (C, lanes 2,3), 0.5 μg (C, lane 1). a, b, c and GLOB : S1 nuclease bands expected for RNA initiated at the natural conalbumin startsite a, the substitute startsites b and c, and the rabbit β globin capsite respectively (see Fig. 1). SP : S1 nuclease bands expected for β globin transcripts initiated upstream from the normal capsite and spliced to a pseudo-acceptor site at +48 (46). FL1 corresponds in size to the length of homology between probe c (Fig. 1) and the transfected recombinants. FL2, FL3 : full length S1 nuclease probes c and a respectively. M : [^{32}P] 5' end-labelled MspI digest of pBR322. The gels contained 8 % acrylamide-50 % urea.

the MLV enhancer, in the direct orientation, stimulates transcription from the major SV40 early-early startsites EES about 10-fold (compare pSV and pSVL, Fig. 4A, lanes 7 and 3, and Table 2). [Transcription is also stimulated from the minor late-early startsites (33) as seen on longer exposition of the autoradiograms (results not shown)]. With the MLV enhancer in the opposite orientation a reproducible lower stimulation is observed : about 12-fold for the conalbumin startsite a (compare pTCT, pTCTL and pTCTLI in Fig. 2A, lanes 1-3, Table 1) and about 6-fold for the SV40 EES

Table I

ACTIVATION OF TRANSCRIPTION FROM THE CONALBUMIN PROMOTER ELEMENT BY THE MLV, MSV AND SV40 ENHANCERS.

RNA synthesis from conalbumin startsite a (%)			
ENHANCER	RECOMBINANT	CELL LINE	
		MOUSE NIH3T3	HUMAN HeLa
NONE	pTCT	1	1
MLV	pTCTL	12 , 20 , 27	8 , 5
	pTCTLI	9 , 15	NI
	pTCTL3	1.3 , 1.4 , 1.7	NI
	pTCTLI3	1.8 , 2.1 , 2.6	NI
	pTCTL37	1 , 1	NI
	pTCTLI37	1 , 1	NI
	pTLCT	5 , 14	NI
	pTLICT	3 , 3	NI
MSV	pTCTS	12 , 15	NI
SV40	pTCTB	670 , 300 , 430	1700 , 3000
	pTCTB3	NI	188 , 212
	pTCTB37	NI	47 , 71
	pTBCT	NI	14 , 5
	pTBICT	NI	61 , 99

The values were obtained from different DNA preparation in independent experiments. They are corrected for transcription from the co-transfected internal control pβ(244+)β (which is not shown in all Figs.) and are expressed relatively to the amount of RNA initiated from pTCT in each cell line. NI = not investigated.

(compare pSV, pSVL and pSVLI, Fig. 4A, lanes 7, 3 and 4, and Table 2). In contrast, the SV40 enhancer, in the direct orientation, stimulates transcription from the conalbumin startsite a about 500-fold (compare pTCT and pTCTB in lanes 1, 4 and 5, Fig. 2A, and Table 1 ; for lane 5 ten times less RNA was used for S1 nuclease mapping than in lanes 1 and 4, whilst for lane 4 only the globin probe e was used) and from the SV40 EES about 70-fold (compare pSV and pSVB in Fig. 4A lanes 7, 2 and 1, and Table 2; ten times less RNA was used for lane 1 than for lanes 2 or 7, whilst for lane 2 only the globin probe e was used). These results show that in NIH 3T3 cells the MLV enhancer stimulates transcription at least twenty times less efficiently than the SV40 enhancer from the conalbumin promoter element and six times less efficiently from the SV40 early promoter.

This smaller stimulation with the MLV enhancer was surprising because

Laimins et al. (20) reported that a similar DNA fragment from the closely related Moloney murine sarcoma virus (MSV) was more efficient than the SV40 enhancer in stimulating gene expression in LMTK- cells. To compare the MLV and MSV enhancers directly, we constructed recombinants with the MSV enhancer fragment immediately upstream from either the conalbumin (pTCTS, Fig. 1) or SV40 early (pSVS1, Fig. 1) promoter elements. Under our conditions, the MSV enhancer stimulates transcription from the conalbumin startsite a approximately 14-fold [compare pTCT and pTCTS in Fig. 2B, lanes 1, 2 and 7 and 8 respectively and Table 1; in Table 1 the results of Fig. 2B were corrected for transcription from the internal control, pβ(244+)β, which was analyzed in a separate experiment and which accounts for the apparently greater difference between pTCTB and pTCTS in Fig. 2B]. The MSV enhancer stimulates transcription from the SV40 EES about 5-fold (compare pSV and pSVS1 in Fig. 4A, lanes 7 and 5, and Table 2). These 14- and 5-fold stimulations with the MSV enhancer are slightly smaller than the 20-and 10-fold stimulations observed in equivalent MLV-enhancer recombinants (compare pTCT, pTCTL and pTCTS, and pSV, pSVL and pSVS1, Table 1). Laimins et al. (20) inserted the MSV enhancer at the SphI (128) site of the SV40 early promoter, so that their recombinants retained part of the SV40 72 bp repeat enhancer sequence between nucleotides 107 and 128. To test whether this could account for the difference with our results we also constructed pSVS2 (Fig. 1) which contains the SV40 early promoter extending up to the SphI site, and the MSV enhancer directly upstream. In this recombinant the MSV enhancer stimulates transcription from the SV40 EES about 8-fold in NIH3T3 cells and 17-fold in $LMTK^-$ cells (Table 2). This stimulation is at most 3-fold greater than the stimulation observed when the shorter SV40 early promoter fragment was used (compare pSVS1 and pSVS2 in Fig. 4A, lanes 5 and 6, and Table 2) and cannot account for the difference between our results and those of Laimins et al. (20) on the relative efficiencies of enhancement by the MSV and SV40 enhancers (see Discussion). Our results show that, under our conditions, the MLV and MSV enhancers are much less efficient than the SV40 enhancer in stimulating transcription from heterologous promoters in mouse cells.

Stimulation of transcription by the MLV enhancer decreases with increasing distances of the enhancer from the conalbumin promoter element.

To determine the efficiency with which the MLV enhancer stimulates transcription when it is dissociated from the conalbumin promoter element, we constructed recombinants containing the enhancer

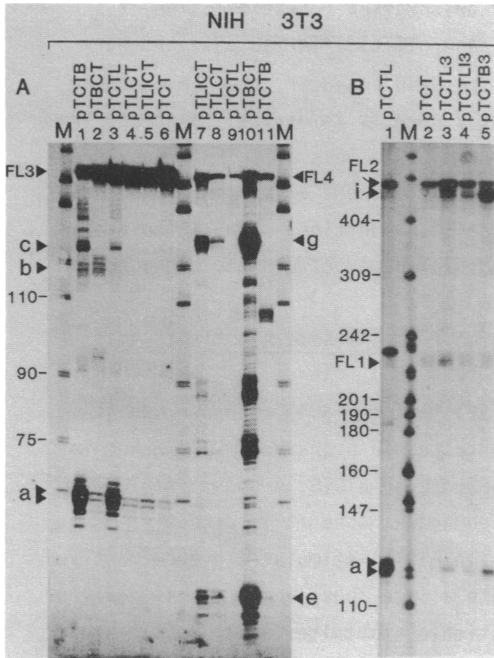

Figure 3 : S1 nuclease analysis of RNA from transfected NIH 3T3 cells.

RNA from transfections with pTCT, pTCTL3, pTCTLI3, pTCTB3, pTLICT, pTLCT and pTBCT was analyzed by S1 nuclease mapping using either probe a (A, lanes 1-6), probe b (A, lanes 7-11), or probe c (B). The amounts of RNA used were : 20 μg (A, lanes 2-5, 7-10), 2 μg (A, lane 1), 60 μg (A, lane 6), 40 μg (B, lanes 1-4), 8 μg (B, lane 5). a, b, c, e, g, i : S1 nuclease band expected for RNA initiated at startsites a, b, c, e, g and i respectively (Fig. 1). FL4 : full length S1 nuclease resistant probe b. FL1-3 and M are as in the legend to Fig. 2. The gels contained either 8 % (part A) or 4 % (part B) acrylamide-50 % urea.

either directly downstream from the promoter element (pTLCT and pTLICT, Fig. 1) or upstream and separated from it by 275 bp (pTCTL3 and pTCTLI3, Fig. 1) or 3737 bp (pTCTL37 and pTCTLI37, Fig. 1) of pBR322. When located 275 bp upstream from the promoter element, the MLV enhancer stimulates transcription about 1.5-fold in the direct orientation (compare pTCT and pTCTL3 in Fig. 3B, lanes 2 and 3, and Table 1), and about 2.2-fold in the opposite orientation [compare pTCT and pTCTLI3 in Fig. 3B, lanes 2 and 4, and Table 1 ; the results in Table 1 were corrected for transcription from the internal control plasmid pβ(244+)β]. Although these stimulations are small, they were reproducibly observed.

When located directly downstream from the promoter element, the MLV enhancer stimulates transcription about 10-fold in the direct orientation (compare pTCT and pTLCT Table 1 and in Fig. 3A, lanes 6 and 4 respectively, 3 times more pTCT than pTLCT RNA was used) and about 3-fold in the opposite orientation [compare pTCT and pTLICT, Table 1, and Fig. 3A, lanes 6 and 5 respectively, 3 times more pTCT than pTCT RNA was used; the results in Table 1 are corrected for transcription from the internal control pβ(244+)β, which was analyzed in a separate experiment]. In contrast to these results, when the MLV enhancer is 3,737 bp upstream from

the conalbumin promoter, and in either orientation, no stimulation of transcription is observed (see pTCTL37 and pTCTLI37 in Table 1, and not shown). These results show that, like the SV40 enhancer, the MLV enhancer stimulates transcription most efficiently when it is directly upstream from the conalbumin promoter element. The enhancer is less efficient when it is either directly downstream from the promoter element or upstream and separated from it by interposed DNA sequences. In addition, stimulation of transcription decreases with increasing distances between the enhancer and the conalbumin promoter element.

The MLV enhancer stimulates transcription from the same substitute startsites as the SV40 enhancer.

We have previously shown (12) that the SV40 enhancer can stimulate transcription from substitute startsites in prokaryotic pBR322 or in conalbumin promoter sequences (sites b and c in pTCTB, e and g in pTBCT, and i in pTCTB3, see Fig. 1). The MLV enhancer, when it is directly upstream from the conalbumin promoter (in pTCTL) stimulates predominantly transcription from the natural startsite a (see above)and proportionally less transcription from the minor substitute startsites b and c (compare pTCTL and pTCT in lanes 3 and 6 of Fig. 3A and lanes 1, 2, 5 and 6 of Fig. 2B). The SV40 enhancer, in the equivalent recombinant pTCTB (see Fig. 1), stimulates transcription in similar relative proportions from the same startsites (Fig. 3A, lane 1). In recombinants containing the SV40 enhancer in the SalI site of pBR322 (see for example pTCTB3, Fig. 1), transcription is stimulated from a substitute startsite, i, at nucleotide 610 in pBR322. Transcription from the equivalent MLV enhancer recombinants (pTCTL3 and pTCTLI3, Fig. 1) was analyzed by S1 nuclease mapping using probe c (see Fig. 1). For pTCTL3, pTCTLI3, as well as for pTCTB3, RNA initiated at the substitute startsite i was detected (Fig. 3B, lanes 3-5), whereas no equivalent RNA was detected for the enhancerless recombinant, pTCT (lane 2 in Fig. 3B). The MLV and SV40 enhancers stimulate transcription to different relative extents from startsites i and a. With the MLV-enhancer recombinants, pTCTL3 and pTCTLI3, similar amounts of RNA are transcribed from startsites i and a (lanes 3,4 Fig. 3B), whereas in the SV40 enhancer recombinant, pTCTB3, more RNA is transcribed from i than a (lane 5, Fig. 3B). The reason for this difference is not understood at present.

When the SV40 enhancer is downstream from the conalbumin promoter (pTBCT, Fig. 1) transcription is stimulated less efficiently from the natural conalbumin startsite a, than from substitute startsites e and g on the other strand (see Fig. 1 and Ref. 12). In the converse situation, with the

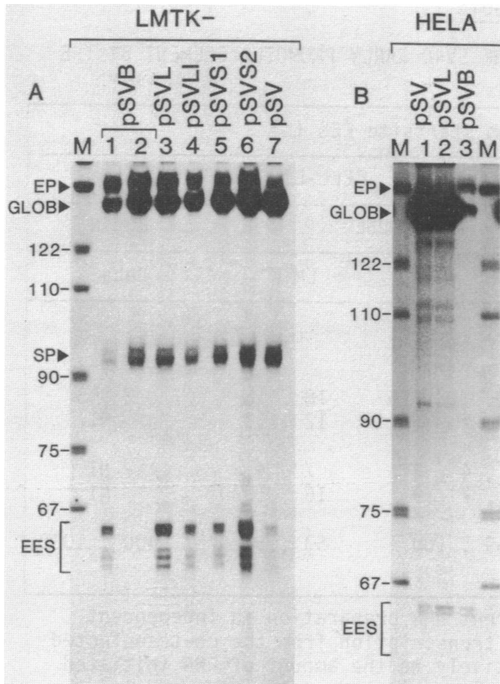

Figure 4 : S1 nuclease quantitation of RNAs from transfected HeLa or LMTK$^-$ cells.
RNA from transfections with pSV, pSVB, pSVL, pSVLI, pSVS1 and pSVS2 in LMTK$^-$ cells (A) or pSV, pSVL and pSVB in HeLa cells (B) was analyzed by S1 nuclease mapping using either a mixture of probes d and e (A, lanes 1, 3-7 ; B, 1-3) or probe c alone (A, lane 2) (Fig. 1). The amounts of RNA used were 2 μg (A, lane 1), 20 μg (A, lanes 2-7), 0.04 μg (B, lane 3) and 20 μg (B, lanes 1, 2). EES : S1 nuclease bands expected for RNA initiated at the SV40 EES startsites (see Fig. 1 and 33). EP corresponds in size to the length of the homology between probe e and the input recombinants other than pβ(244+)β (see probe e for pSVB in Fig. 1, A) and is a measure of the total amount of RNA initiated upstream from the sequence discontinuity (33, 27). SP and GLOB are as in the legend to Fig. 2. The gels were 8 % acrylamide-50 % urea.

enhancer upstream from the conalbumin promoter element, no transcription is detected from the substitute startsites, e and g. Hence there appears to be a preferential stimulation of RNA transcription away from, rather than towards, the enhancer (see Ref. 12). RNA from transfections with pTLCT and pTLICT, with the MLV enhancer downstream from the conalbumin promoter and in either orientation, was analyzed by S1 nuclease mapping with the conalbumin non-coding strand probe b (Fig. 1). RNA initiated from e and g startsites was detected for both pTLCT and pTLICT as well as for pTBCT. However, the MLV enhancer is less efficient than the SV40 enhancer in stimulating transcription from these startsites (compare lanes 7, 8 and 10, Fig. 3A). With pTLCT and pTBCT, approximately equal amounts of RNA were detected starting at sites e and g (Fig. 3A, lane 8, 10 and results not shown), whereas for pTLICT and pTBICT about twice as much RNA starting from site g compared to site e was detected (Fig. 3A, lane 7, results not shown and Ref. 12). In contrast, no transcription was detected from these startsites either with recombinants with the enhancers upstream from the promoter element (pTCTL and pTCTB, Fig. 3A, lanes 9 and 11, a negligibly small

Table II

ACTIVATION OF TRANSCRIPTION FROM THE SV40 EARLY PROMOTER ELEMENT BY THE MLV, MSV AND SV40 ENHANCERS

RNA synthesis from startsite EES (%)				
		CELL-LINE		
		MOUSE		HUMAN
ENHANCER	RECOMBINANT	NIH3T3	LMTK⁻	HeLa
NONE	pSV	1	1	1
MLV	pSVL	11 , 11 , 11 , 10	18 , 15	2 , 3
	pSVLI	4 , 7 , 7 , 6	12 , 13	NI
MSV	pSVS1	5 , 4 , 4 , 3	7 , 4	NI
	pSVS2	6 , 11 , 7 , 9	16 , 18	NI
SV40	pSVB	53 , 59 , 100	53 , 53	400 , 1000

The values were obtained from different DNA preparation in independent experiments. They are corrected for transcription from the co-transfected internal control and expressed relatively to the amount of RNA initiated from pSV in each cell line. NI = not investigated.

amount of transcription was detected for startsite e of pTCTB) or in an enhancerless recombinant (pTCT, not shown). A comparison of the amounts of RNA starting from sites a (see above) with those from e and g suggests that the MLV enhancer preferentially stimulates transcription reading away from the enhancer. These results show that the MLV enhancer stimulates transcription from the same substitute startsites as the SV40 enhancer, and that the pattern of stimulation is very similar for both enhancers.

Activation of transcription by the MLV enhancer is cell-type specific.

Various enhancers, including that of MSV, have been reported to be tissue and species-specific (see Discussion). To show that the MLV enhancer stimulates specific transcription in a cell-line specific way, we transfected recombinants containing the MLV enhancer directly usptream from either the conalbumin or SV40 early promoter elements in either mouse (NIH 3T3 or LMTK$^-$) or human cells (HeLa). The MLV enhancer stimulates transcription from the conalbumin startsite a about 7-fold in HeLa cells and about 20-fold in NIH 3T3 cells (compare pTCT and pTCTL in Table 1), whereas the SV40 enhancer stimulates specific conalbumin transcription about 2,500-fold in HeLa cells, and about 500-fold in NIH 3T3 cells (compare pTCT and pTCTB

in Table 1). The SV40 enhancer is thus about 350 times more efficient than MLV in stimulating conalbumin transcription in human HeLa cells (compare pTCTB and pTCTL in lanes 1 and 3 of Fig. 2C, one hundred times less pTCTB RNA was used for lane 1 than pTCTL RNA for lane 3), whereas the SV40 enhancer is only about twenty-five times more efficient in NIH3T3 cells (compare pTCTB and pTCTL in lanes 1 and 3, Fig. 2A, ten times less pTCTB RNA was used for lane 1 than pTCTL RNA for lane 3). Similar results are obtained with the SV40 early promoter element. The MLV enhancer, when it is directly upstream from the SV40 early promoter, stimulates transcription from EES about 2-3-fold in HeLa cells (compare pSV and pSVL in lanes 1 and 2, Fig. 4B, and Table 2), 11-fold in NIH 3T3 cells (see Table 2) and 17-fold in LMTK$^-$ cells (compare lanes 7 and 3 of Fig. 4A, and Table 2). The SV40 enhancer stimulates SV40 early transcription about 700-fold in HeLa cells (compare pSV and pSVB, lanes 1 and 3, Fig. 4B, five hundred times less pSVB RNA was used for lane 3 than pSV RNA for lane 1, and Table 2), about 70-fold in NIH 3T3 cells (Table 2), and about 53-fold in LMTK$^-$ cells (compare pSVB and pSV, Fig. 4A lanes 1 and 7, ten times less pSVB RNA was used and Table 2). These results show that, for stimulation of transcription from either the conalbumin or SV40 early promoter elements, the MLV enhancer is more efficient in mouse cells, and conversely, the SV40 enhancer is more efficient in HeLa cells. However, even in mouse cells, the SV40 enhancer is still about twenty-five times more efficient than the MLV enhancer in stimulating transcription from the conalbumin promoter element, and, depending on the mouse cell-line used, about 3-10 times more efficient for the SV40 early promoter element.

DISCUSSION

To extend our previous studies on the SV40 enhancer to another enhancer, we have compared directly, in similar recombinants, the MLV and SV40 enhancers. Our present results show that, despite the facts that MLV enhancer is weaker than the SV40 enhancer, and has a different cell-type specificity, the properties of the two enhancers are remarkably similar.

The MLV enhancer is less efficient than the SV40 enhancer in activation of specific transcription.

We have consistently observed that the MLV enhancer is about 25 times less efficient than the SV40 enhancer in stimulating transcription in mouse cells. This was found with both the conalbumin and the SV40 early promoter elements (compare pTCT, pTCTB and pTCTL in Table 1, and pSV, pSVB

and pSVL in Table 2), and in two mouse fibroblast cell lines (NIH 3T3 and LMTK$^-$) in which the virus is infectious (34, 35). The weaker stimulation also does not appear to depend on the particular conditions of transfection. When we decreased the quantity of either pSVB or pSVL DNA in the transfection (by adding carrier pBR322 DNA so as to keep the quantity of DNA transfected constant), the amount of specific transcription decreased proportionally for both recombinants (not shown). These results suggest that under our conditions of transfection there is an excess of transcription factors over DNA in the cell and that the amount of specific transcription detected really reflects the efficiency of the particular enhancer-promoter element combination. In agreement with this finding, the same relative stimulations were observed by the MLV and SV40 enhancers in pSVL and pSVB whether or not they were cotransfected with the internal control pβ(244+)β [containing the polyoma enhancer which is most active in mouse cells] (not shown and Ref. 9).

Our results on the efficiency of activation by the MLV enhancer are surprising because Laimins et al. (20) reported that the enhancer from the closely related retrovirus MSV is more efficient than the SV40 enhancer in stimulating gene expression when transfected in mouse LMTK$^-$ cells. Since the MLV and MSV enhancers differ by several point mutations, deletions and nucleotide insertions (23, 24), including one nucleotide change in the "core"-sequence of Weiher et al. (36), we compared both enhancers for their efficiency of stimulation of transcription from both the conalbumin and SV40 early promoter elements. We find that the MSV enhancer is slightly weaker than the MLV enhancer, even when we use the same SV40 early promoter containing DNA fragments as Laimins et al. (20) (see Results). Since Laimins et al., (20) have reported that the MSV enhancer is twice as efficient as the SV40 enhancer in LMTK$^-$ cells, and we find that in the best case the MSV enhancer is three times less efficient than the SV40 enhancer (pSVS2 and pSVB, Fig. 1 and Table 2), there remains a six-fold difference in the measured efficiencies of the two enhancers, which is unexplained at the present time. Under our conditions the MLV and MSV enhancers are weaker than the SV40 enhancer for stimulation of transcription in mouse cells.

The MLV and SV40 enhancers are cell-line specific.

We have shown that the MLV enhancer stimulates transcription from both the conalbumin and SV40 early promoter elements about five times more efficiently in two mouse fibroblast cell lines (NIH 3T3 and LMTK$^-$) than in

a human cell line (HeLa) (compare pTCT and pTCTL in Table 1, and pSV and pSVL in Table 2). The converse specificity is observed for the stronger SV40 enhancer, which stimulates transcription from the heterologous promoter elements about 5-10 more efficiently in human cells than in mouse cells (compare pTCT and pTCTB in Table 1, and pSV and pSVB in Table 2). These results show that the MLV enhancer stimulates transcription most efficiently in the homologous cell lines, in which MLV is normally infectious. Several other viral and cellular enhancers have been shown to be most active in the species in which the virus is infectious (9, 19, 20) or in the tissues in which the gene is normally active (37-42).

The properties of the MLV 75 base pair repeat are very similar to those of the SV40 enhancer.

The MLV enhancer shares a number of properties with the extensively characterised SV40 enhancer. The MLV enhancer, in either orientation just upstream from two heterologous promoter elements, stimulates transcription initiating from the natural startsite (see above). It still stimulates transcription when it is separated from the conalbumin promoter element by 275 bp of upstream pBR322 sequences, however, as we have observed with the SV40 enhancer (12), this stimulation is about 10-fold lower than when the enhancer is in close apposition to the promoter element (compare pTCTL, pTCTLI, pTCTL3 and pTCTLI3, Fig. 1 and Table 1). Increasing the enhancer-promoter distance, with 3737 bp of upstream pBR322 sequences (pTCTL37 and pTCTLI37, Fig. 1 and Table 1) results in a further decrease in activation, such that stimulation of transcription is no longer detectable. This is not surprising; taking into account that the MLV enhancer is less efficient than the SV40 enhancer, and that in the equivalent SV40 enhancer containing recombinants (see pTCTB and pTCTB37, and Ref. 12) transcription activation is reduced about 100-fold, then the expected activation by the MLV enhancer would be unmeasurably small. The MLV enhancer is also less efficient in stimulating transcription when it is directly downstream from the promoter element (compare pTCTL, pTCTLI, pTLCT adn pTLICT, Table 1 and Fig. 1).

The MLV enhancer also stimulates transcription to similar relative extents from the same substitute conalbumin and pBR322 startsites (b, c, e, g and i, Fig. 1) as the SV40 enhancer (see Results). In addition, our results show that the MLV and SV40 enhancers stimulate RNA transcribed away from the enhancer in preference to that transcribed towards the enhancer. However, we cannot exclude that this effect may only be apparent, and may

be related to the experimental conditions (12). In contrast, some small differences are observed between the two enhancers. In pTCTL3 and pTCTLI3 (Fig. 1) less RNA is detected initiated at startsite i than a, whereas with the SV40 enhancer containing recombinant pTCTB3 (Fig. 1) the converse is true (see lanes 3-5, Fig. 3B). Similarly, whereas the SV40 enhancer is always slightly more efficient in one of its orientations (12), the MLV is slightly more efficient in the direct orientation when it is directly upstream or downstream from the conalbumin promoter element (see pTCTL, pTCTLI and pTLCT, pTLICT in Table 1), and more efficient in the opposite orientation when it is further upstream (see pTCTL3 and pTCTLI3 in Table 1). However, despite these small differences, it is clear that the SV40 and MLV enhancers stimulate transcription in a similar characteristic manner.

Recently, Laimins et al. (21) have used the indirect chloramphenycol acetyltransferase (CAT) assay to compare the properties of the SV40 and MSV enhancers on both natural and heterologous promoter elements. Although their results concerning the bidirectionality of the MSV enhancer are in good agreement with our present data concerning the MLV enhancer, they have reported a much lower "effect of distance" on the activity of the MSV enhancer. Whether this reflects a difference between the closely related MSV and MLV enhancers or is due to the use of the CAT assay which does not indicate where RNA is actually initiated is unknown.

A common mechanism to account for the potentiator effects of the SV40 and MLV enhancers.

We have previously reported that the SV40 enhancer activates proximal promoter elements in preference to more distal ones (7, 12). To account for these results, we proposed that the SV40 enhancer may act as an "entry-site", so that a component of the transcription machinery, "enters" the template on the 72 bp repeat region and then tracks the DNA in either direction to find sequences which promote transcription. In agreement with this model de Villiers et al. (44) and Kadesh and Berg (45) have shown that the distal (with respect to the SV40 enhancer) of two tandemly arranged genes was poorly expressed, but the expression of the same distal gene was enhanced when the promoter of the proximal gene was inactivated. More recently we have shown that the "distance effect" on activation of transcription by the SV40 enhancer is apparently biphasic (13). Interposing relatively short (more than 200 bp) fragments of DNA between the enhancer and SV40 early or conalbumin promoter elements leads to a drastic 95 % decrease in activation. Further insertions lead to a less dramatic

decrease in enhancement, such that the enhancer stimulates transcription to a similar extent when separated by 650 or 3700 bp from the activated promoter elements. We have speculated that the short range effect which is critically dependent on "distance" and which cannot be accounted for by the presence of interposed promoter elements, may either reflect the existence of an additional promoter element in the SV40 DNA fragment which contains the SV40 enhancer or be an intrinsic property of enhancer elements (13). However, we show here that the MLV enhancer shows the same short range distance dependence of activation observed with the SV40 enhancer. Interposing the same short DNA fragments between either enhancer and the conalbumin promoter element, leads to a similar decrease in activation of transcription. This suggests that the short range distance dependence of activation is an intrinsic property common to all enhancers. The properties of the MLV enhancer, and in particular the facts that activation of transcription decreases with the distance of a promoter element from the enhancer, and that the same proximal substitutes startsites are activated as with the SV40 enhancer, suggest that the MLV enhancer may also act as an entry site for some component(s) of the transcription machinery.

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*To whom reprint requests should be sent

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