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A transcriptional enhancer with specificity for erythroid cells is located in the long terminal repeat of the Friend murine leukemia virus

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We investigated the ability of the U3 region of the long terminal repeats (LTR) of the Friend murine leukemia virus (Fr-MuLV) and Moloney murine leukemia virus (Mo-MuLV) to promote transcription in a variety of human cell lines. Our analysis reveals the presence of a transcriptional enhancer with specificity for erythroid cells in the U3 region of the Fr-MuLV. This constitutes the first example of an enhancer with such a property. Analysis of the Mo-MuLV enhancer suggests that it is active at least in erythroid and lymphoid cells and has thus a less restricted specificity than the Fr-MuLV enhancer. The different tissue specificities of the two enhancers correlate with the different tissue selectivities and pathogenic properties of the two viruses.

Key words: long terminal repeat/murine leukemia virus/transcription enhancer/erythroid cells

Introduction

The Moloney murine leukemia virus (Mo-MuLV) induces T-cell lymphomas after injection into newborn NFS mice, whereas the Friend helper murine leukemia virus (Fr-MuLV) induces primarily erythroleukemia (Tambourin *et al.*, 1979; Oliff *et al.*, 1981; Silver and Fredrickson, 1983). The construction of recombinant viruses demonstrated the existence of a strong determinant of the distinct disease specificities of the two viruses within the U3 region of their long terminal repeats (LTR) (Chatis *et al.*, 1983, 1984). Since this region contains the putative viral transcription control elements, including the transcriptional enhancer (Coffin, 1982; Laimins *et al.*, 1982, 1984; Koch *et al.*, 1984; Clark *et al.*, 1985), these results suggested that the disease specificity might be determined, at least in part, by tissue-specific regulation of viral transcription. Such a direct relationship between tropism or disease specificity and cell type specificity of the viral enhancer has been established for several viruses (Celander and Haseltine, 1984; Herbomel *et al.*, 1984; Shaul *et al.*, 1985; Yoshimura *et al.*, 1985; Weber and Schaffner, 1985).

These results led us to analyse the ability of the U3 region of the Fr-MuLV genome to promote transcription in a variety of human cell lines, including different types of haematopoietic cells lines. We find that the Fr-MuLV transcriptional control elements show striking preferential activity in erythroid cells. This preferential activity is due, at least in part, to the specificity of an enhancer located in the U3 region. While no activity of the Fr-MuLV enhancer is detected in lymphoid cells, our data suggest that the Mo-MuLV enhancer is active in T-cells. Thus, the difference in disease specificity of the two viruses may be explained in part by the different tissue specificities of their transcriptional enhancers.

Results

To evaluate the ability of Fr-MuLV sequences to promote transcription in different cell lines, we used the chloramphenicol acetyltransferase (CAT) system developed by Gorman and collaborators (1982a): the CAT gene is placed under the control of the putative transcripitonal control element and the construct is transfected into cells in culture. After allowing for expression, the enzymatic activity is measured in cell extracts by estimation of the conversion of [^{14}C]chloramphenicol into its acetylated form. This value is taken as a measure of the transcriptional activity of the transcriptional control element (Laimins *et al.*, 1984; Gorman *et al.*, 1982b; Celander and Haseltine, 1984; Herbomel *et al.*, 1984; Hamada *et al.*, 1984).

Cell type-specific expression from the Fr-MuLV LTR

In a first series of experiments, we measured the relative expression level of Fr-MuLV- and SV40-driven CAT constructs in different human cell lines as well as in the mouse 3T6 fibroblast cell line. The structure of the Fr-MuLV–CAT plasmid, pF-CAT, is shown in Figure 1. In this plasmid, the CAT gene is placed downstream to the entire putative Fr-MuLV transcription control region contained in a *Bam*HI–*Kpn*I DNA fragment. This fragment carries an imperfect direct repeat of 65/74 bp which, by analogy with other retroviruses, might constitute the viral enhancer (Koch *et al.*, 1984; Wolff *et al.*, 1985; Clark *et al.*, 1985). In addition, it contains the entire putative promoter and the transcription initiation site (Figure 1).

To take into account possible variations in transfection efficiencies, we used a double parallel transfection procedure: (i) the pF-CAT plasmid was transfected in parallel with two plasmids: pSV2-CAT and pA10-CAT2, which carry the CAT gene under the control of the entire SV40 early transcription control region and of the same region deleted of the functional transcription enhancer, respectively (Figure 1). The CAT gene has been shown to be expressed efficiently from the pSV2-CAT plasmid in a variety of cell lines (Gorman *et al.*, 1982a; Mosthaf *et al.*, 1985), while the pA10-CAT2 plasmid expresses only very low levels of the enzyme (Laimins *et al.*, 1984); (ii) each cell line to be tested was transfected in parallel with HeLa cells as a reference, i.e. the same DNA–calcium phosphate precipitate or DNA–DEAE-dextran solution was applied to both cell types.

For each cell line we define a relative CAT expression level as the ratio of the enzymatic activities obtained with the Fr-MuLV construct and the pSV2-CAT plasmid. From this, a specificity index (SI) was derived as the ratio of the relative expression levels obtained with the test and reference cell lines. While the relative expression level appears to vary between independent experiments, we find more constant values for the SI. The SI thus provides an estimate of the preferential expression of the Fr-MuLV-driven construct over the SV40-driven construct, in the test cell line compared with the reference cell line. Figure 2 shows a representative CAT assay after transfection of pF-CAT and pSV2-CAT into HeLa cells, 3T6 cells, and the erythroid cell lines K562 and HEL (Lozzio and Lozzio, 1975; Anderson *et al.*, 1979; Martin and Papayannopoulou, 1982). Quantitation of this experi-

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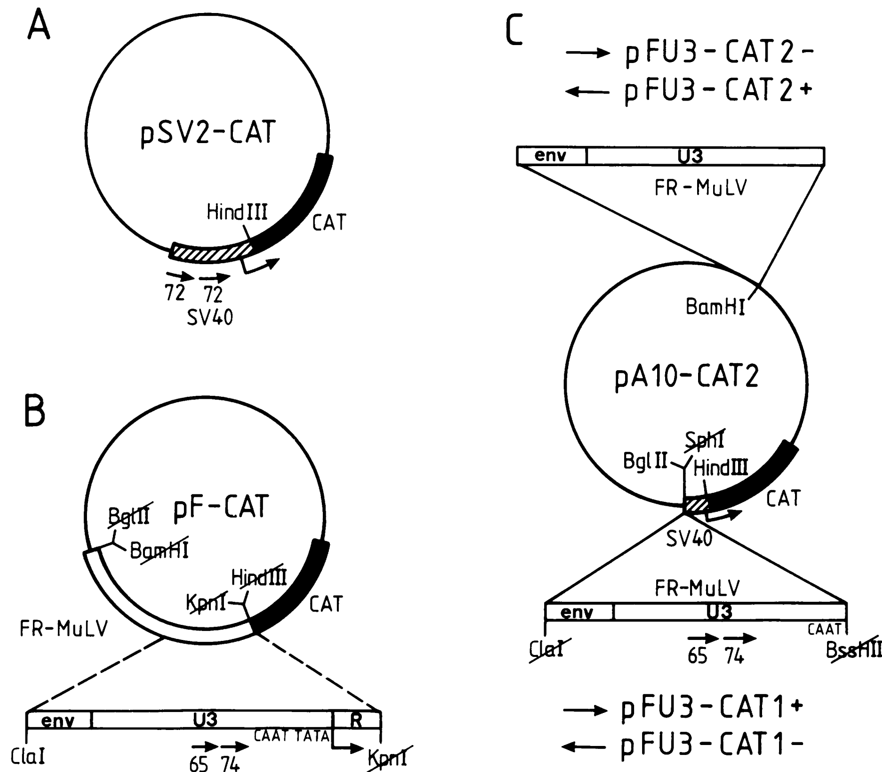

Fig. 1. Structure of the plasmids used in the transfection experiments. (**A**) pSV2-CAT contains the CAT gene under the control of the entire SV40 early transcriptional control region (Gorman *et al.*, 1982a). (**B**) pF-CAT was constructed by recombination of a *Bam*HI–*Kpn*I DNA fragment containing the 3′ U3 region of the Fr-MuLV genome, with a *Bgl*II–*Hin*dIII DNA fragment from the CAT plamsid pA10-CAT2 (Laimins *et al.*, 1984). (**C**) The four plasmids pFU3-CAT1+, pFU3-CAT1−, pFU3-CAT2+ and pFU3-CAT2− were derived from the enhancerless plasmid pA10-CAT2 by insertion either into the *Bgl*II site or into the *Bam*HI site and in either orientation of a *Cla*I-*Bss*HII DNA fragment containing the putative Fr-MuLV enhancer. The crossed sites were destroyed during the constructions.

ment as well as of others involving different human cell lines is presented in Table I.

We find that with the non-erythroid cell lines, which include fibroblast, hepatoma and B-cells, the SI is of the order of 1, indicating that the relative expression from the Fr-MuLV- and SV40-driven constructs is similar in these cell lines and in the reference cervical carcinoma HeLa cell line (Table I). In contrast, with the erythroid cell lines, we find a much higher SI: ~80 in the case of K562 and ~16 in the case of HEL (Table I). This last figure is a minimum estimate since in some experiments the CAT activity from HEL cells transfected with pSV2-CAT was not above background. Thus, our results indicate a highly preferential expression of the CAT gene in the K562 and HEL cell lines versus the other cell lines tested, when it is under the control of the Fr-MuLV regulatory region, compared with an S40-driven construct.

An enhancer with specificity for erythroid cells

Our results might be explained by preferential transcription from the Fr-MuLV LTR in the erythroid cell lines, preferential transcription from the SV40 regulatory region in the other cell lines or both. Furthermore, if cell-specific transcription occurs from the Fr-MuLV LTR, the determinants of the specificity might reside in the enhancer, in the promoter or in another transcriptional control element not yet identified. To address these questions and to delineate more precisely the cell type specificity of the transcription control region, we measured the enhancer activity in various cell lines of a 0.6-kb *Cla*I–*Bss*HII DNA fragment containing the Fr-MuLV putative enhancer (Figure 1). This fragment is not expected to contain a functional promoter, since it does not include the TATA box and the transcription initiation site (Figure 1). Four plasmids were derived from the enhancerless plasmid pA10-CAT2 by insertion of the *Cla*I–*Bss*HII fragment in both orientations into two different restriction sites, immediately upstream or 4 kb upstream of the SV40 promoter (Figure 1). These constructs were transfected into K562 and 3T6 cells in parallel with pA10-CAT2 and pSV2-CAT as described earlier. An example of a CAT assay is presented in Figure 3. In the erythroid cell line K562, the four different plasmids containing the *Cla*I–*Bss*HII fragment give rise to similar levels of CAT activity, 25–60 times higher than the level obtained with pA10-CAT2 (Table II and data not shown). In 3T6 cells, the presence of the Fr-MuLV fragment enhances the enzymatic activity by only 2- to 10-fold, while the presence of the SV40 enhancer leads to a 50- to 120-fold increase (Table II and data not shown). These results indicate that the *Cla*I–*Bss*HII fragment does contain a functional enhancer since in K562 cells it enhances CAT gene expression by ~50-fold, irrespective of its position and orientation. Furthermore, the weak enhancing activity in 3T6 cells suggests that the Fr-MuLV enhancer is cell type specific. We tested this possibility by transfecting in parallel one of the Fr-MuLV enhancer-containing plasmids, pU3-CAT2− (see Figure 1), with pA10-CAT2 and pSV2-CAT into K562 cells, HeLa cells and the human cell lines listed in Table II. Three erythroid cell lines were used: K562, HEL and PUTKO. PUTKO is a cell hybrid derived from the K562 line and has retained its erythroid characteristics (Klein *et al.*, 1980; Rutherford *et al.*, 1981b). In these three erythroid cell lines, the Fr-MuLV enhancer construct is two to

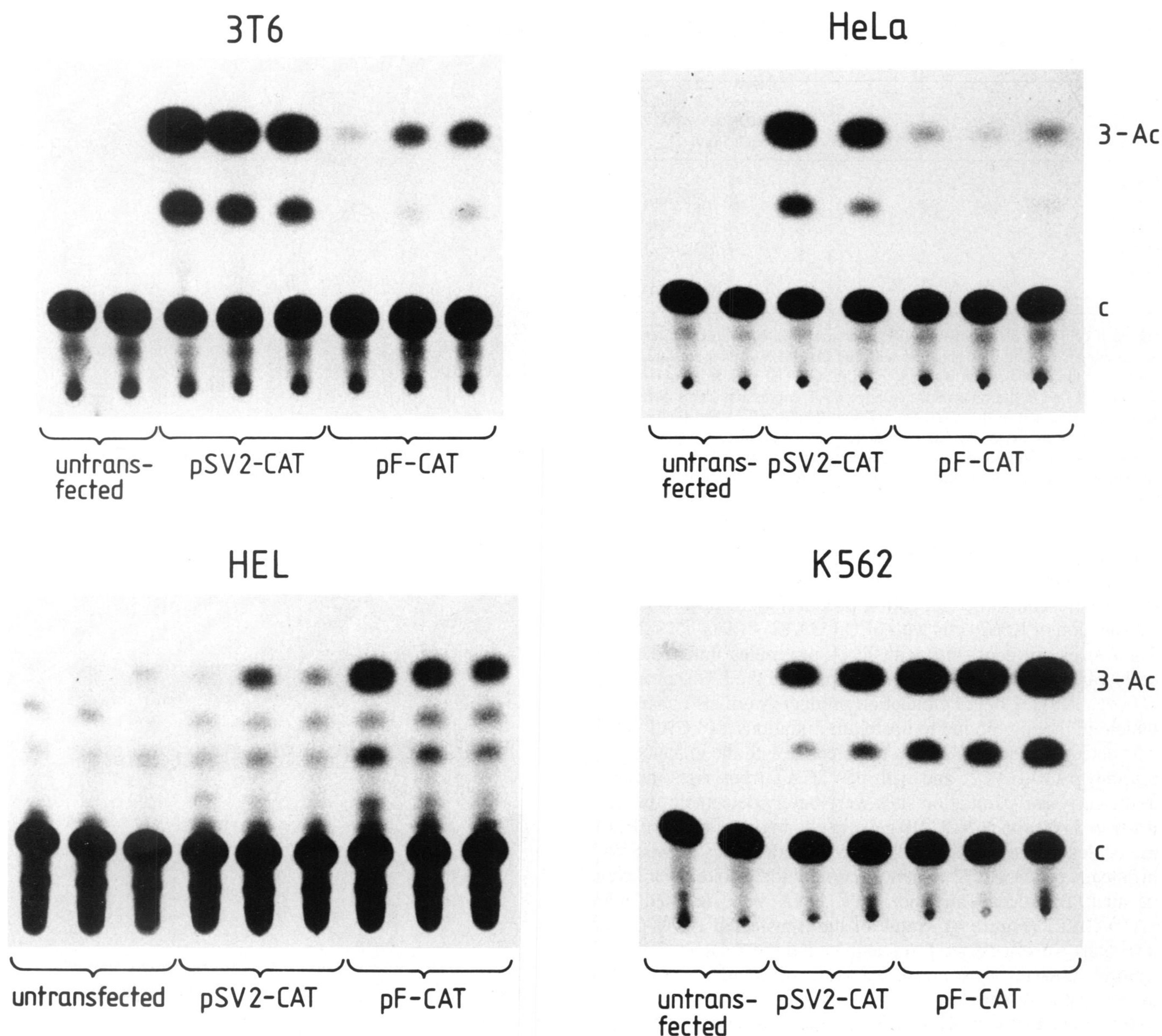

Fig. 2. CAT assays from 3T6, HeLa, K562 and HEL cells, untransfected or transfected with the SV40-driven CAT plasmid pSV2-CAT, or with the Fr-MuLV U3 region-driven CAT plasmid, pF-CAT (see Figure 1 for the structure of the plasmids). For each cell line, and each plasmid, two or three plates were treated in parallel, and the CAT activities were assayed separately from each plate. 'C' corresponds to the unreacted substrate [^{14}C]chloramphenicol and '3-AC' to the major acetylated form (in position 3).

three times more active than the SV40 enhancer construct in directing CAT synthesis (Table II). The presence of the Fr-MuLV enhancer leads to enhancements in CAT expression of ~50- and 150-fold over the pA10-CAT2 level in K562 and PUTKO cells, respectively (Table II). The enhancement is much more limited in HEL cells, presumably due to poor transfectability of this cell line. In all the other cell lines, which include myeloid, B- and T-cell lines, we find that the presence of the Fr-MuLV enhancer leads to only weak increases, if any, in CAT activity over the pA10-CAT2 level, while the presence of the SV40 enhancer activates expression by 20- to 300-fold (Table II). With any of these cell lines, the enhancement obtained with the SV40 enhancer is at least 10-fold higher than that obtained with the Fr-MuLV enhancer (Table II).

In conclusion, the preferential expression observed from the Fr-MuLV LTR in the erythroid cell lines appears to be due, at least in part, to cell type specificity of an enhancer located in the U3 region. Our data suggest that the activity of this enhancer is restricted to erythroid cells.

CAT expression level reflects mRNA level

To verify whether the observed differential expression of the CAT gene was reflecting differences at the RNA level, we quantitated the relative amount of CAT RNA present in transfected cells. RNA was extracted from PUTKO and 3T6 cells, after parallel transfection with pSV2-CAT, pFU3-CAT2− and pA10-CAT2. RNA samples were used to perform a primer extension experiment with a 30-base synthetic oligodeoxynucleotide complementary to the first 30 bases of the CAT mRNA coding sequence (Alton and Vapnek, 1979; Ciliberto *et al.*, 1985). The products of the extension reaction were analysed by polyacrylamide gel electrophoresis (Figure 4). With RNA extracted from PUTKO

Table I. Specificity index of the Fr-MuLV LTR-driven construct pF-CAT in different cell lines

Cell line	Specificity index pF-CAT/pSV2-CAT cell line/HeLa
HeLa	1
K562	80 (38–99)
HEL	>16 (16–16)
BJA-B	1.5 (0.9–2)
HepG2	1.1 (0.2–2)
3T6	0.6 (0.5–0.9)

For each cell line, the relative CAT expression level is defined as the ratio of the enzymatic activities obtained with the Fr-MuLV-driven plasmid, pF-CAT, and the SV40-driven plasmid, pSV2-CAT. The specificity index for a defined cell line is the ratio of the relative CAT expression levels obtained in this cell line and in the reference cell line (HeLa cells). The figures shown are the means of the values obtained in at least three independent experiments involving duplicate or triplicate plates, as described in the legend of Figure 2. The figures in parenthesis are the extreme values obtained.

cells transfected with pFU3-CAT2−, we observed two major elongation products of ~120 and 125 nucleotides, respectively (Figure 4). The same elongation products were observed after transfection of K562 cells with pFU3-CAT2− (data not shown). Their sizes are consistent with RNA molecules initiated at the early-early initiation sites of transcription of the SV40 promoter (Tooze, 1981). Longer elongation products were also observed, which might correspond to upstream initiations. No CAT RNA was detected in PUTKO cells transfected with the enhancerless plasmid pA10-CAT2, and with pSV2CAT transcripts initiated at the early-early initiation sites were barely detectable. In contrast, in 3T6 cells pSV2CAT gave rise to high amounts of RNA molecules initiated at the early-early initiation sites of the SV40 promoter, pFU3-CAT2− gave rise to 20- to 30-fold lower levels of such transcripts and no CAT RNA was detected with pA10-CAT2 (Figure 4). Some of the transfected PUTKO and 3T6 cells were used for CAT assays and the relative CAT activities observed were consistent with the relative CAT RNA levels (data not shown).

In conclusion, our data indicate that in pFU3-CAT2−, the *Cla*I−*Bss*HII DNA fragment derived from the Fr-MuLV genome activates transcription from the SV40 promoter and thus carries a transcriptional enhancer. They demonstrate that the levels of CAT enzyme observed in PUTKO or 3T6 cells transfected with pFU3-CAT2-, pSV2CAT or pA10-CAT2 reflect the respective levels of CAT mRNA and therefore suggest that the differential CAT expression observed in various cell lines from the three constructs is indeed due to differential transcription of the gene.

The Fr-MuLV and Mo-MuLV enhancers have different cell type specificities.

To investigate whether the different disease specificities of Fr-MuLV and Mo-MuLV might reflect differences in the cell type specificites between the respective enhancers, we have evaluated the activity of the Mo-MuLV enhancer in four representative cell lines: HeLa cells, K562 and PUTKO cells and the T-cell line JM-1 (Schneider *et al.*, 1977). We constructed the plasmid pMU3-CAT1−, which is identical to the plasmid pFU3-CAT1− described in Figure 1 except that the *Cla*I−*Bss*HII DNA fragment carrying the viral enhancer was derived in this case from the Mo-MuLV genome. The two plasmids were transfected in parallel with pA10-CAT2 and pSV2CAT into the four cell lines

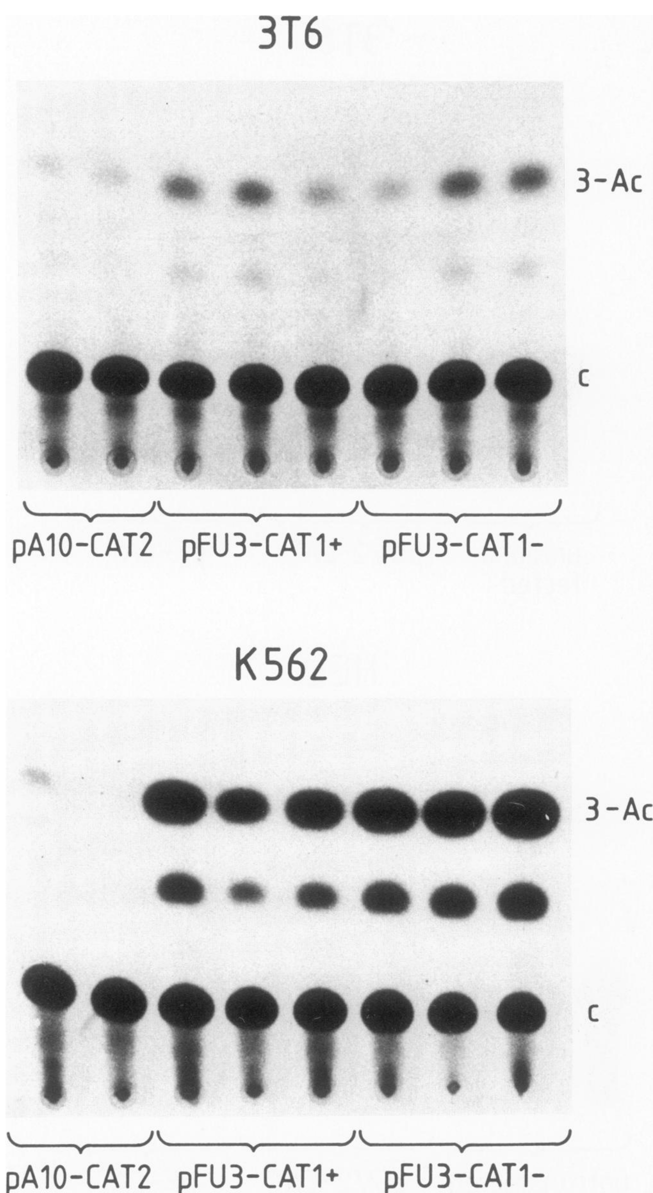

Fig. 3. CAT assays from 3T6 and K562 cells transfected with the enhancerless plasmid pA10-CAT2 or its two derivatives containing the Fr-MuLV enhancer fragment inserted in the *Bgl*II site (see Figure 1 for the structure of the plasmids). The procedure was as described in Figure 2.

and CAT activities were measured. The result of a typical experiment is shown in Figure 5. Evaluation of the activities of the different enhancers was performed as described earlier and is presented in Table III. As noted before, we find that the SV40 enhancer is active in the four cell lines. The Fr-MuLV enhancer is active only in K562 and PUTKO cells, while the Mo-MuLV enhancer is active in K562, PUTKO and JM-1 cells. This result indicates that the Fr-MuLV and Mo-MuLV enhancers have different cell type specificities and suggests that the Mo-MuLV enhancer is active in lymphoid T-cells in addition to erythroid cells.

Discussion

Preferential activity of the Fr-MuLV enhancer in erythroid cell lines

Here, we demonstrate the existence within the U3 region of the

Table II. Fr-MuLV and SV40 enhancer activities in different cell lines

Cell line	Origin	Fr-MuLV enhancer activity	SV40 enhancer activity
Erythroid			
K562	Chronic myeloid leukemia	52 (13–156)	33 (9–170)
PUTKO	K562-P3HR-1 cell hybrid	159 (130–200)	68 (54–83)
HEL	Erythroleukemia	3 (1–5)	2 (1–3)
Myeloid-monocytic			
U937	Histocytic lymphoma	2 (0.8–3)	17 (5–34)
KG-1	Acute myelogenous leukemia	1.5 (1–2)	37 (9–65)
HL-60	Acute promyelocytic leukemia	2 (1–4)	21 (3–40)
Lymphoid			
BJA-B	Burkitt's lymphoma	1.5 (1–2)	16 (6–21)
P3HR-1	Burkitt's lymphoma	7 (5–9)	238 (111–333)
JM-1	Acute T-cell lymphoblastic leukemia	1.5 (1–2)	56 (35–73)
MOLT-4	Acute T-cell lymphoblastic leukemia	1 (1–1)	19 (4–34)
Non-hematopoietic			
HepG2	Hepatoma	2.5 (1–4.5)	80 (29–150)
HeLa	Cervical carcinoma	1.5 (1–2)	78 (19–295)
HFF	Foreskin fibroblasts	0.8 (0.5–1)	11 (3–18)
3T6	Mouse fibroblasts	5 (3–11)	79 (44–120)

The enhancer activity of the Fr-MuLV or SV40 enhancer is defined for each cell line as the ratio of CAT activities obtained with the enhancer-containing plasmid, pFU3-CAT2– or pSV2-CAT, respectively, and the enhancerless plasmid pA10-CAT2. The figures shown are the means of the values obtained in at least two independent experiments, involving duplicate or triplicate plates, as described in the legend of Figure 2. The figures in parenthesis are the extreme values obtained.

LTR of the Fr-MuLV of a transcriptional enhancer element with preferential activity in human erythroid cell lines compared with other human hematopoietic and non-hematopoietic cell lines. The enhancer activity was evaluated using a system in which the CAT gene is driven by an enhancerless SV40 early promoter. We find that the Fr-MuLV U3 region only weakly enhances the expression of the CAT gene in the non-erythroid cell lines tested. In any of these cell lines, the SV40 enhancer is at least 10 times more active. By contrast, in the erythroid cell lines K562 and PUTKO, the presence of the Fr-MuLV U3 region on the CAT plasmid enhances CAT expression by factors of ~50- and 150-fold, while the SV40 enhancer is 2–3 times less active. In addition, in the other human erythroid cell line tested, HEL, we also observed a higher activity of the Fr-MuLV enhancer compared with the SV40 enhancer. In this case, however, the enhancement observed is very limited, presumably due to the poor efficiency of transfection of this cell line. Preferential activity of the Fr-MuLV enhancer in HEL cells is also suggested by the preferential expression in these cells observed for the plasmid pF-CAT carrying the entire Fr-MuLV transcription control region.

Tissue-specific enhancers

The nature of the cell lines K562 and HEL have been questioned. The cell line K562 was established from a pleural effusion of a patient with chronic granulocytic leukemia in terminal blast crisis (Lozzio and Lozzio, 1975). It was originally thought to represent a primitive granulocyte cell. This view was based mainly on morphological examination, the absence of lymphoid surface makers and the presence of granulocyte antigens (Klein *et al.*, 1976; Lozzio *et al.*, 1976; Drew *et al.*, 1977). However, these studies did not prove that K562 cells were myeloid precursor cells, and later studies demonstrated biochemically that K562 cells do show an erythroid phenotype (Anderson *et al.*, 1979; Rutherford *et al.*, 1979, 1981a; Hoffman *et al.*, 1981). The HEL cell line was established from a patient with Hodgkin's disease, who later developed erythroleukemia (Martin and Papayannopoulou, 1982). It was shown to have erythroid biochemical markers (Martin and Papayannopoulou, 1982). However, it was later demonstrated that treatment of HEL cells with 12-O-tetradecanoyl-phorbol-13 acetate (TPA) induces changes that are characteristic of macrophage-like cells, suggesting that the cell line might have a bipotential nature (Papayannopoulou *et al.*, 1983). In view of these data, the preferential activity of the Fr-MuLV enhancer in K562 and HEL cells could be related to the potential myeloid or monocytic nature of these cell lines, as well as to their erythroid nature. However, the enhancer was not found to be active in the myeloid or monocytic cell lines tested which do not present any erythroid characteristics. Taken together, our observations suggest that the Fr-MuLV enhancer is preferentially active in erythroid cells or in a subpopulation of erythroid cells. The Fr-MuLV enhancer would then constitute the first example to our knowledge of an enhancer with such a specificity. Cellular and viral tissue-specific enhancer elements have been described, with specificity for either lymphoid, pancreatic or haematopoietic cells (Gillies *et al.*, 1983, 1984; Neuberger, 1983; Banerji *et al.*, 1983; Walker *et al.*, 1983; Queen and Stafford, 1984; Mosthaf *et al.*, 1985). Tissue-specific enhancers are thought to play an important role in the regulation of gene expression during cell differentiation and development. In the case of the immunoglobulin heavy chain enhancer, the existence of tissue-specific factors able to interact with the enhancer has also been demonstrated (Ephrussi *et al.*, 1985; Mercola *et al.*, 1985). The Fr-MuLV enhancer might constitute a model for cellular erythroid cell-specific enhancers. Furthermore, its existence suggests the presence of specific *trans*-acting factors, either in erythroid cells or in non-erythroid cells, able to modulate its activity. Such factors would be expected to regulate also the activity of putative cellular erythroid cell-specific enhancers, functionally homologous to the Fr-MuLV enhancer.

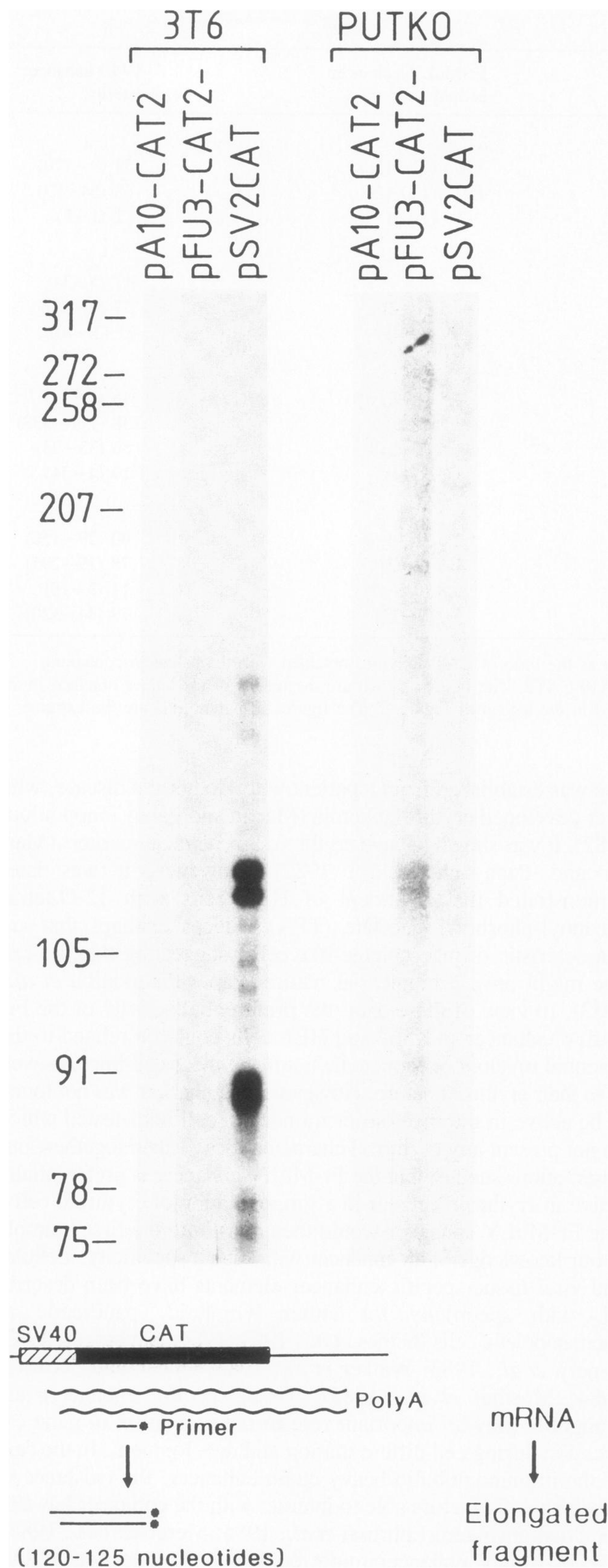

Fig. 4. CAT RNA primer extension assay. RNA was extracted from PUTKO and 3T6 cells transfected with either pA10-CAT2, pFU3-CAT2− or pSV2-CAT. The products of the extension experiment, obtained after hybridization to these RNAs of a 30-mer synthetic oligodeoxynucleotide complementary to the coding region, were analysed by electrophoresis on a 6% polyacrylamide−urea denaturing gel.

Table III. Comparison of the activities of the Mo-MuLV and Fr-MuLV enhancers in HeLa, K562, PUTKO and JM-1 cells

Cell line	Fr-MuLV enhancer activity	Mo-MuLV enhancer activity	SV40 enhancer activity
K562	18 (17−18)	27 (24−31)	5 (4−6)
PUTKO	165 (130−200)	147 (128−165)	68 (54−83)
JM-1	1.3 (1−1.6)	22 (22−22)	8 (5−10)
HeLa	1.1 (1.3−0.9)	1.2 (1.8−0.7)	52 (46−57)

For each cell line, we calculated the ratio of CAT activities obtained with the enhancer-containing plasmid, pMU3-CAT1−, pFU3-CAT1− or pSV2-CAT, and the enhancerless plasmid pA10-CAT2. The figures shown are the means of the values obtained in two independent experiments involving duplicate or triplicate plates, as described in the legend of Figure 2. The actual values are shown in parenthesis.

Enhancer involvement in pathogenesis

We carried out a limited comparative study of the specificities of the Fr-MuLV and Mo-MuLV enhancers. The two enhancers are similarly active in erythroid K562 and PUTKO cells and both are inactive in HeLa cells. In the T-cell line JM-1, however, the Mo-MuLV enhancer is active, while the Fr-MuLV enhancer is not. Furthermore, according to our preliminary data, a similar difference is observed in the B-cell line P3HR-1 (Hinuma *et al.*, 1967) (data not shown). Thus, our results indicate that the specificity of the Mo-MuLV enhancer is less stringent than that of the Fr-MuLV enhancer. In particular, they suggest that the Mo-MuLV enhancer is active in lymphoid cells. A correlation is observed between the pathogenic properties of the two viruses in NSF mice, and the specificity of the respective enhancers: the leukemia originates from a cell type in which the enhancer is active. Thus, activity of the viral enhancer in a particular cell type might be a necessary condition for the induction of a leukemogenic process in this cell type. However, this is clearly not sufficient: the Mo-MuLV enhancer is active in erythroid cells and possibly in other non-T hematopoietic cells and, nevertheless, the virus gives rise almost exclusively to lymphomas. Other factors are thus involved in the determination of the disease specificity. The contribution of the envelope gene has been demonstrated (Oliff *et al.*, 1984) and Chatis and collaborators have also proposed that the U3 region may determine the type of virus that is generated in the course of an infection and this, in turn, may determine the type of disease (Chatis *et al.*, 1983).

Enhancer activity in non-hematopoietic cells

The Moloney murine sarcoma virus (MSV) and the Mo-MuLV have almost identical nucleotide sequences within the U3 region of their genomes (van Beveran *et al.*, 1982). We have analysed the activity of the MSV enhancer in HeLa, K562, PUTKO, P3HR-1 and JM-1 cells and observed that the enhancer behaves like the Mo-MuLV enhancer in these cells (data not shown). The two enhancers have also been studied in several mouse, rat and primate cell lines and found to be active in different fibroblast cell lines (Laimins *et al.*, 1982, 1984; Jolly *et al.*, 1983; Augereau and Wasylyk, 1984; Linney *et al.*, 1984; Graves *et al.*, 1985; Clark *et al.*, 1985; Schulze *et al.*, 1985). In particular, it was observed that while the MSV enhancer is more active than the SV40 enhancer in mouse fibroblast L cells, it is much less active in human HeLa cells or in monkey CV-1 cells (Laimins *et al.*, 1982). These data were interpreted as an indication of species specificity of the two enhancers. In view of our results, indicating in particular that the Mo-MuLV enhancer is more active than the SV40 enhancer in human erythroid and T-cell lines, the data

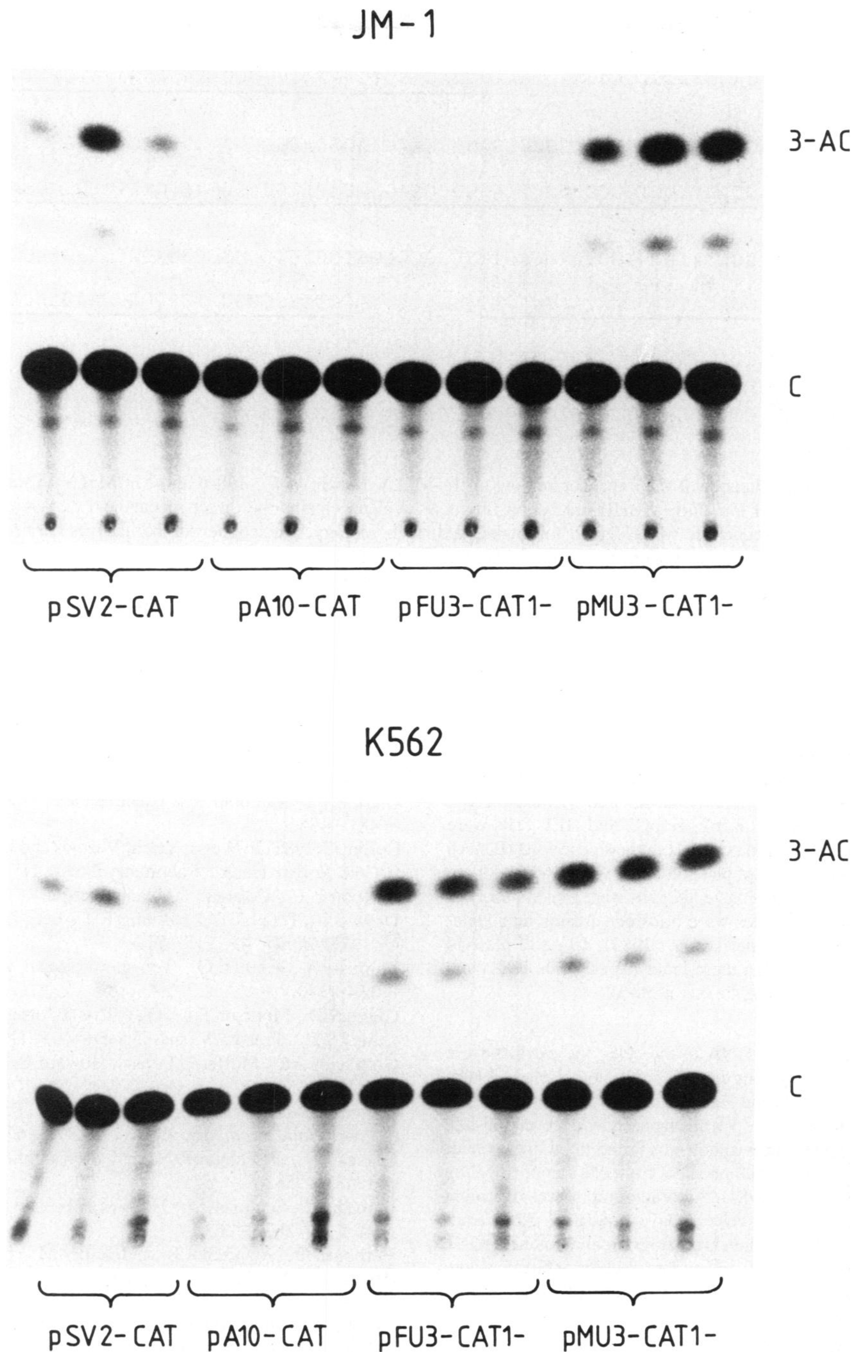

Fig. 5. CAT assays from erythroid K562 and lymphoid JM-1 cells transfected with pSV2-CAT, the enhancerless plasmid pA10-CAT2 or its two derivatives, pFU3-CAT1− or pMU3-CAT1−, which carry respectively the Fr-MuLV or the Mo-MuLV enhancer. The procedure was as described in Figure 2.

of Laimins and collaborators should be re-interpreted as a manifestation of the cell type specificity of the MSV enhancer. The activity of the Mo-MuLV and MSV enhancers in fibroblast cell lines might be a consequence of the history of forced passage of the viruses in this type of cell. The same reason might explain the weak activity of the Fr-MuLV enhancer that we observed in mouse fibroblast 3T6 cells. Clark and collaborators have analysed the properties of the Fr-MuLV LTR using a genetic selection assay in a rat fibroblast cell line (Clark *et al.*, 1985). Although their assay does not allow direct quantitation of transcriptional activity, their data are consistent with transcriptional activation by the Fr-MuLV enhancer in these cells.

In conclusion, we have shown that the Fr-MuLV and Mo-MuLV enhancers have different cell type specificities, which correlate with the pathogenic phenotype of the viruses. The nucleotide sequences of the enhancer regions of the two viruses are highly homologous (Koch *et al.*, 1984, and Figure 6). It should thus be possible to map by directed mutagenesis the putative element within the enhancers which is responsible for the differential activities. This element might constitute a binding site for a tissue-specific *trans*-acting factor. We are currently investigating this possibility.

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Fr-MuLV -352 GATCAAGGTCAGGTACA.CGAAAACAGCTAACGTTGGGCCAAACAGGATATCTGTGGTAA
Mo-MuLV -354 GATCAAGGTCAGGAACAGATGGAACAGCTGAATATGGGCCAAACAGGATATCTGTGGTAA

        -293 GCAGTTTCGGCCCCGGCCCGGGGCCAAGAACAGAT.........ACGCTGGGCCAAACA
        -294 GCAGTTCCTGCCCCGGCTCAGGGCCAAGAACAGATGGAACAGCTGAATATGGGCCAAACA

        -243 GGATATCTGTGGTAAGCAGTTTCGGCCCGGTCGGCCCCGGCCCGAGGCCAAGAACGGATG
        -234 GGATATCTGTGGTAAGCAGTT.........CCTGCCCCGGCTCAGGGCCAAGAACAGATG

        -183 GTCCCCAGATATGGCCCAACCCTCAGCAGTTTCTTAA
        -183 GTCCCCAGATGCGGTCCAGCCCTCAGCAGTTTCTAGA
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Fig. 6. Comparison of the nucleotide sequences of the enhancer regions of Fr-MuLV (Koch *et al.*, 1984) and Mo-MuLV (Shinnick *et al.*, 1981). The sequences compared correspond to part of the *Cla*I−*Bss*HII restriction fragment, which contains the direct repeats and carries the enhancer activity (Augereau *et al.*, 1984, and data not shown). The sequences were aligned to obtain maximal homology. Nucleotide numbering was carried out with respect to the respective viral RNA capping sites. The 75-bp direct repeats of the Mo-MuLV are underlined.

Materials and methods

Cell culture and DNA transfection

3T6, HeLa, K562, HepG2 (Knowles *et al.*, 1980) and human foreskin fibroblast cells were maintained in Dulbecco's modified Eagle's (DME) medium with 10% fetal calf serum (FCS). The other cell lines were maintained in RPMI 1640 medium with 10% FCS. HEL cells were transferred into DME medium with 10% FÇS 1 day before transfection. We used adherent sublines of K562 and HEL cells, which were checked for globin gene expression (Charnay and Maniatis, 1983, and unpublished observation). 3T6, HeLa, K562, HepG2 and HEL cells were transfected by exposure to a DNA−calcium co-precipitate as described (Banerji *et al.*, 1981; Treisman *et al.*, 1982). 20 μg of plasmid DNA were used per 9 cm plate. Five hours after the addition of the precipitate, the cells were glycerol shocked (25% glycerol for 90 s). The other cell lines were transfected using an adaptation of the DEAE−dextran procedure (Banerji *et al.*, 1983). 10 μg of plasmid DNA were used for $5 \times 10^6 - 10^7$ cells. In these latter experiments HeLa and K562 cells were also transfected according to this protocol.

CAT assay

Forty four hours after the addition of the DNA to the cells, the extracts were prepared and the CAT activity was assayed as described (Herbomel *et al.*, 1984). 25 μl of extract were used with 3T6 cells and HeLa cells and 75 μl with the other cell lines. The reaction was carried out for 1−3 h adding acetyl-CoA every 45 min as described (Herbomel *et al.*, 1984). The reaction was found to be linear under these conditions. Separation of acetyl chloramphenicol from chloramphenicol by t.l.c. was as described (Herbomel *et al.*, 1984). Autoradiography was overnight on Kodak XAR-5 films, except with HEL cells (2 days exposure). After autoradiography, the spots were cut out from the chromatogram and counted. CAT activity was expressed as the percentage of conversion of chloramphenicol into its 3-acetyl form. The SI and enhancer activities were calculated from data obtained within the same experiment.

RNA extraction and primer elongation

Total cellular RNA from transfected 3T6 and PUTKO cells was prepared as described previously (Treisman *et al.*, 1982, 1983). The oligodeoxynucleotide primer was labelled at the 5′ end with [^{32}P]ATP and polynucleotide kinase. Primer elongation was performed as described previously (D'Onofrio *et al.*, 1985). Relative levels of transcripts were estimated by scanning the autoradiograms with a densitometer.

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