

Promoter activity of the 1731 *Drosophila* retrotransposon in a human monocytic cell line

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The resemblance between retrotransposons and retroviruses suggests an evolutionary relationship and indicates that they may share common transcription factors. We have analyzed the behaviour of the *Drosophila* 1731 retrotransposon promoter in the human monocytic U937 cell line. We show that the long terminal repeat (LTR) of 1731 promotes CAT (chloramphenicol acetyl transferase) activity in these cells, in which it is enhanced by phorbol esters. Using gel mobility assays, we detected a human nuclear protein that binds in the U3 region of the LTR in a sequence-specific manner. Its precise target was determined by a DNase I footprinting experiment.

Retrotransposon 1731; *Drosophila melanogaster*; U937; Chloramphenicol acetyl transferase

1. INTRODUCTION

Retrotransposons are mobile genetic elements integrated in the genome of all eukaryotes examined so far as, for example, the Ty elements of yeast, the copia element of *Drosophila*, the intracisternal type A particles of rodents and the THE1 repeats of humans (see [1–5] for reviews). Their similarities with retroviruses, as well as the mutations they may induce, justify increasing development of their study.

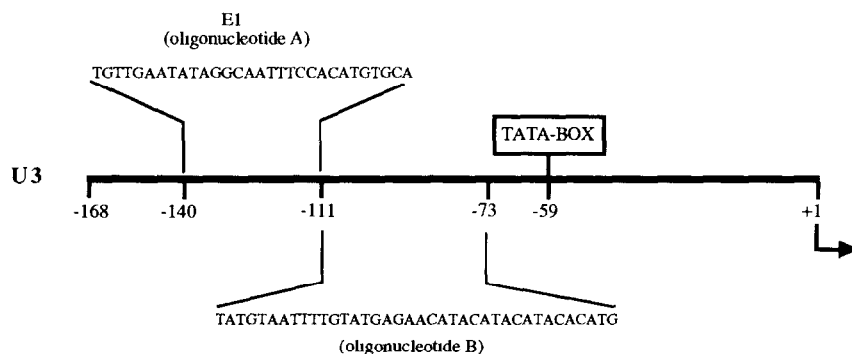
Retrotransposons are usually classified into two groups with regard to the functional organization of their pol gene [6,7]. 1731 is a *Drosophila melanogaster* retrotransposon which belongs to the Ty1-copia group [8,9]. It is 4,648 bp long and moderately repeated in the genome of the fly as well as in *Drosophila* cultured cells. The two 336 bp long terminal repeats (LTRs) of 1731 exhibit the typical U3-R-U5 architecture of provirus LTRs, and the internal sequence encodes a gag and a pol gene. Expression of the pol gene in bacteria has shown that it encodes an authentic reverse transcriptase activity [10]. As with other retrotransposons of the Ty1-copia group, a typical env gene was not detected in the 1731 element. The unidirectional transcription of 1731 is promoted by the 5' LTR and generates a major polyadenylated RNA corresponding to the whole element. 1731 is an especially attractive model for a molecular analysis of regulatory mechanisms because of its dual and opposite regulation by heat shock and steroids

[9,11,12]: 1731 expression has indeed been shown to be induced by heat shock and repressed by 20-hydroxyecdysone (20-OH), the steroid hormone controlling insect molts. A functional analysis of the 5' LTR has revealed three different parts [11]: the 'core promoter' (positions –110 to +89 with respect to the initiation of transcription), followed by a silencer (positions +90 to +168), and preceded by a transcriptional activator (positions –168 to –111). This last fragment includes two imperfect direct repeats, and the more proximal one with respect to the TATA box strongly activates the core promoter. This short segment (–140 to –111), named E1, was shown to be involved in hormone and heat-shock regulation [11,12].

The striking resemblance between retrotransposons and retroviruses suggests some evolutionary link between them, and it was assumed that retroviruses may have derived from retrotransposons by the mere acquisition of a functional env gene or vice versa [13]. Recent developments of PCR methods allowed a systematic search for retrotransposons through a number of genomes, using conserved sequences of the reverse transcriptase domains [14–18]. New retrotransposons with high levels of similarity were thus found in very distant species. One disconcerting example is the existence of a retrotransposon very akin to 1731 in the herring, *Clupea harengus* [14]. The persistence of sequence homology in such distant species, despite the fact that reverse transcription usually introduces numerous errors, seems to plead in favor of horizontal transmission [6,7,14,15,17,19]. These works approach the evolutionary history of retrotransposons considering the conservation of the reverse transcriptase domain. We assumed that, if horizontal transmissions have really occurred, the transcrip-

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A -



B -

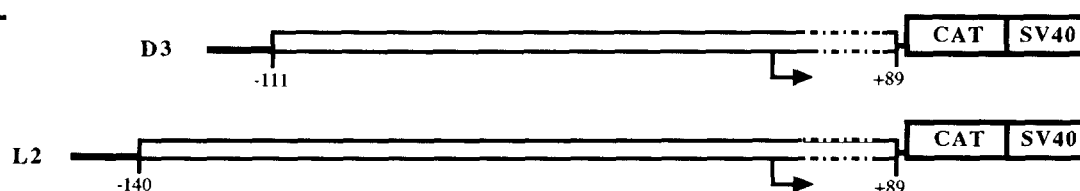


Fig. 1 (A) Schematic representation of the 1731 LTR U3 region. +1 refers to the transcription start. The sequence of the A and B oligonucleotides used in the gel retardation experiments is specified. (B) Schematic representation of the pCAT12/1731 subclones used in transfection assays [11]. PolyA SV40, simian virus 40 polyadenylation site.

tional and translational machinery of the recipient host had to be efficient on the exogenous element, and it is therefore expected that traces persist at the functional level. It would mean that targets for ubiquitous transactivators might be conserved. It can be illustrated, for instance, by the fact that 1731 LTRs display several putative targets for vertebrate DNA binding factors. The U3 part of LTR contains sequences similar to the glucocorticoid receptor binding site [9,11] and to the NF κ B binding site [11]. This led us to think that some 1731 promoter activity might be kept even in distant animal species.

In the present paper, we analyze the behavior of the 1731 promoter after transfer to human monocytes. Our results show that a heterologous promoter, coming from a species as distant from human as is the fly, *Drosophila*, remains able to use for its benefit the host transcriptional and translational machinery. We also characterized a nuclear monocytic factor that binds sequence-specifically the U3 region of the 1731 LTR.

2. MATERIALS AND METHODS

2.1. Plasmids

The plasmids D3 and L2, were used throughout these experiments [11]. Briefly, D3 contains the 1731 core promoter (positions -110 to +89) subcloned into the pCAT12 vector. A synthetic oligonucleotide corresponding to positions -140 to -111 of the LTR (i.e. E1, the proximal repeat that has a strong activator role on the 1731 core promoter) was inserted upstream of the core promoter in D3, generating plasmid L2 (Fig. 1). The pCMV-CAT plasmid [20], a kind gift of Dr N. Israël, was used as the transfection control.

2.2. Cell culture and transfection

The human histiocytic lymphoma cell line, U937, a morphologically monocyte-like cell line (a kind gift of Prof. D. Zagury) was used [21]. Cells were grown in RPMI 1640 supplemented with fetal calf serum (5% v/v), L-glutamine (2 mM) and antibiotics (penicillin 100 IU/ml; streptomycin 100 μ g/ml). Transfections were performed by electroporation (Cellect; Eurogentec) [22]. $30 \cdot 10^6$ cells were centrifuged ($1,000 \times g$; 5 min) and resuspended in RPMI 1640 without fetal calf serum. They were then transferred in an electrophoresis cuvette and 30 μ g of supercoiled DNA was added. A simple pulse (capacitance, 1,800 μ F; voltage, 250 V; pulse time, 40–50 ms) was applied. The cells were then transferred to 10 ml of growth medium and incubated at 37°C/5% CO₂ before being collected 40 h later. Phorbol ester treatment was performed in some experiments by adding 50 ng/ml of PMA (phorbol 12-myristate 13-acetate; Sigma) and 5 μ g/ml of PHA (phytohemagglutinin; Sigma) 20 h before collecting the cells [23].

2.3. Chloramphenicol acetyl transferase (CAT) assays

CAT assays were performed as previously described [11]. Protein concentrations were determined by the method of Bradford using the Bio-Rad protein assay. 10–20 μ g of proteins (1731 plasmids) or 0.5 μ g of proteins (CMV plasmid) were used for the assay. The enzymatic reaction was stopped after 4 h and chloramphenicol and its acetylated forms were separated by thin layer chromatography. Results were expressed as the percentage of the acetylated form per mg of protein per h.

2.4. Oligodeoxynucleotides and gel retardation assays

The oligonucleotides A (upper strand: 5'-TGTGTAATATAGGCAATTTCCACATGTGCA-3') and B (upper strand: 5'-TATGTAATTTGTATGAGAACATACATACATACATG-3') corresponding to positions -140 to -111 and -110 to -73 of the 1731 LTR, respectively, were used in gel retardation experiments. Nuclear extracts, heparin agarose chromatography, and DNA-protein binding assays were performed as previously described [24] using double-

stranded sonicated herring sperm DNA as the non-specific competitor.

2.5. DNase I footprinting

The binding reaction was performed as described above and care was taken that enough protein extract was used for complexing all the probe. After addition of an equal volume of 5 mM CaCl₂/10 mM MgCl₂, DNase I digestion was carried out by adding 1 µl of DNase I (Boehringer) as indicated in the legend of the figure. After 1 min at room temperature, the reaction was stopped by the addition of 2.5 µl of 250 mM EDTA. The samples were ethanol-precipitated and loaded on a 15% acrylamide/urea sequencing gel.

3. RESULTS

3.1. Activity of the 1731 promoter in human cells

To determine whether the 1731 core promoter could be functional in human cells, we first realized transfections of D3 plasmid in U937 cells by electroporation. The CMV-CAT plasmid was used as a transfection efficiency control in all the experiments.

It was clearly shown that the 1731 core promoter is able to promote CAT (chloramphenicol acetyl transferase) activity in human U937 cells. The CAT activity induced by the 1731 core promoter is about 400-fold lower than that of CMV. These results allow us to conclude that the LTR of the invertebrate retrotransposons

1731 is able to keep its promoter function in a monocyte-like human cell line.

We also analyzed the effect of the 28 bp activator repeat, E1, on the level of CAT activity. An average of a 3-fold enhancement of the 1731 core promoter in the presence of this short sequence (plasmid L2) was observed, suggesting that DNA binding factors may interact with this segment of the LTR to enhance CAT expression.

Phorbol esters have been well known to be mitogenic agents that activate a large range of transcription factors. We tested the level of CAT activity in the presence or absence of PHA and PMA mitogens. The CAT activity of the D3 and L2 plasmids was significantly induced by PHA/PMA (20 h treatment). An average of 5-fold and 10-fold enhancement by phorbol ester treatment was obtained with D3 and L2, respectively.

These results are summarized on Fig. 2. They suggest that transactivating factor(s) activated by phorbol esters can modulate the 1731 promoter in human cells.

3.2. In vitro binding of U937 cell proteins to the 1731 LTR

Since the 1731 promoter can induce a CAT activity

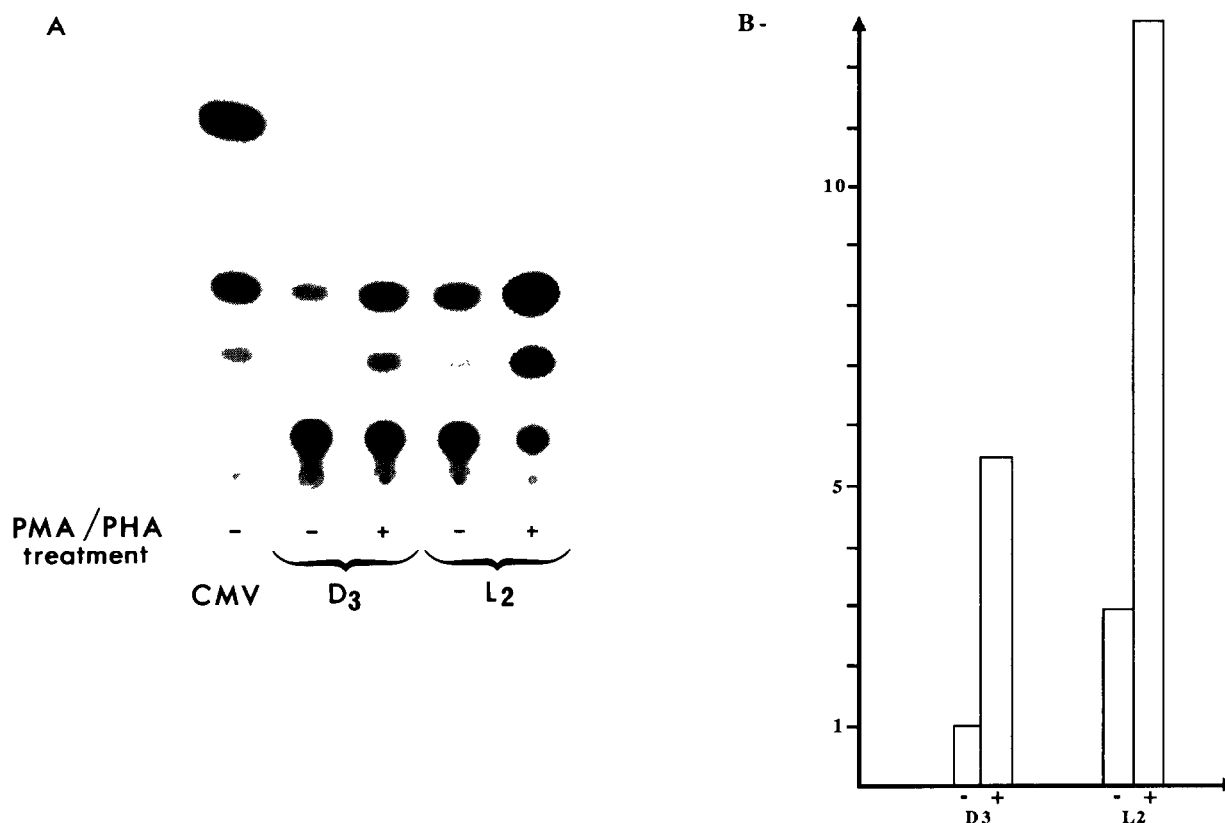


Fig. 2. Transfection of D3 and L2 subclones of 1731 in U937 cells. (A) Autoradiogram corresponding to CAT assays from a representative transfection with (+) or without (-) PMA/PHA treatment. (B) Diagram representing the average of six independent experiments. CAT assays were performed 40 h after transfection and CAT reactions were allowed to continue for 2 h with 0.05 µg or 10 µg of protein for CMV-CAT or 1731-CAT plasmids, respectively. The relative CAT activity was calculated by normalizing the percent conversion of chloramphenicol to its acetyl derivatives taking arbitrarily the results obtained for D3 without treatment as equal to 1.

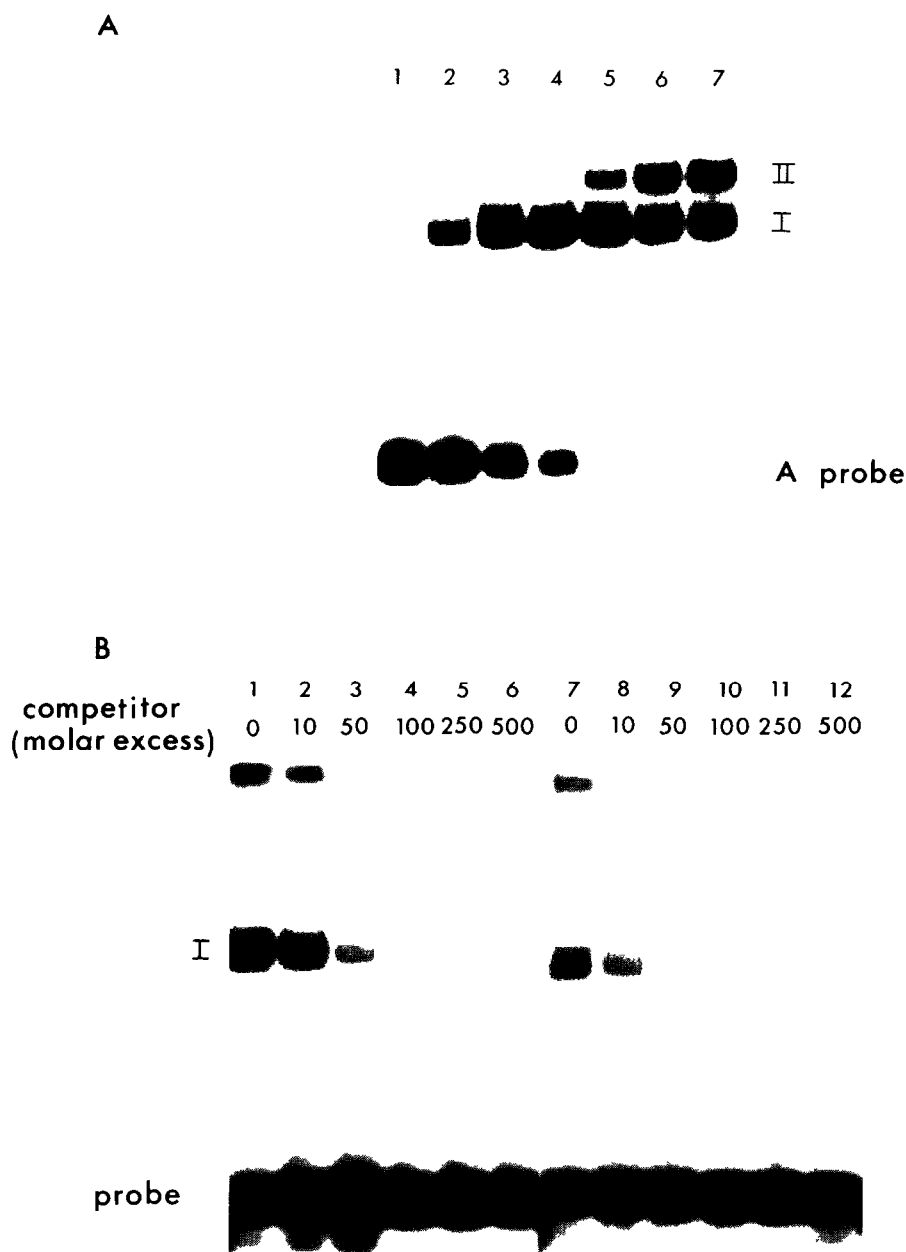


Fig. 3. Gel retardation assays using the A and B oligonucleotides as probes with U937 cell nuclear extracts. (A) Detection of complexes I and II with the A probe. The protein concentration was increased from 0.5 to 3 μg per 23 μl . The same pattern was obtained with the B oligonucleotide as a probe (not shown). (B) Self-competition experiments using 1 μg of protein. Molar excesses of competitor were added as indicated. Lanes 1-6, A probe; lanes 7-12, B probe.

in human U937 cells, and is sensitive to phorbol ester treatment, we looked for DNA binding protein(s) able to bind, in a sequence-specific manner, the 1731 promoter upstream sequences. For this purpose, we used in gel retardation assays several oligodeoxynucleotides covering the region located upstream of the TATA box. Two oligonucleotides named A (i.e. E1; including positions -140 to -111) and B (covering positions -110 to -73) were used throughout these experiments (see Fig. 1).

Nuclear extracts were prepared from U937 human cells and assayed for DNA binding activity. They con-

tain a factor that retards the movement of the probe through the gel and gives rise to two retarded bands, I and II, with both oligonucleotides A and B (Fig. 3). Increasing the concentration of proteins in the gel-shift assay reveals the faster migrating band then the slower one, which suggests that I and II might correspond to monomer and dimer forms of the same proteic factor (Fig. 3A). To test the specificity of the interaction, binding competition analyses were performed. The retarded complexes were abolished in the presence of an excess of unlabeled self-competitor: 50% competition was ob-

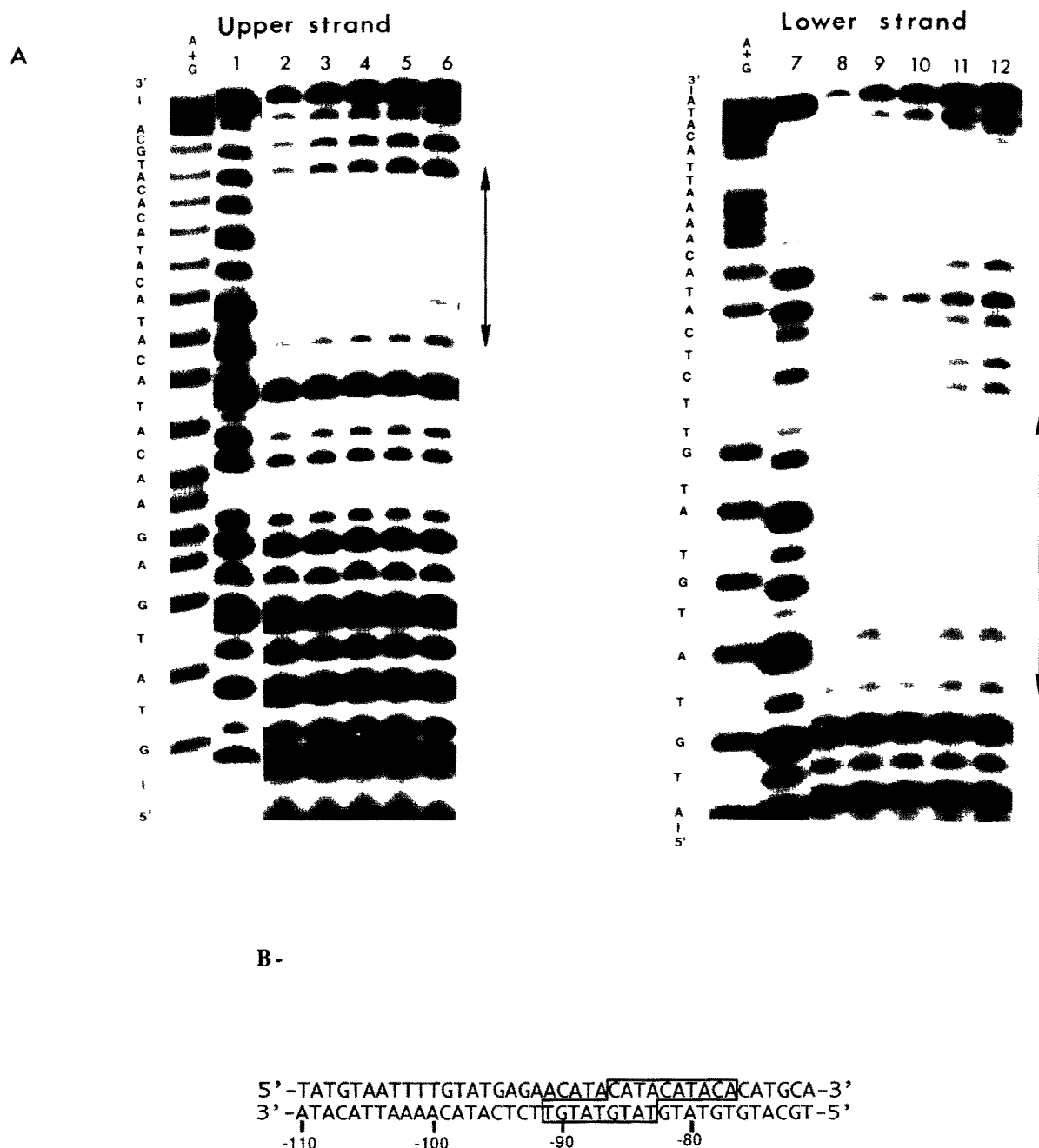


Fig. 4. DNase I protection analysis. (A) Protection of oligonucleotide B with factor 0.3 (i.e. eluted in the 0.3 M KCl fraction of the heparin-agarose column). Both strands of the oligonucleotide were alternately labeled as indicated. A+G, Maxam and Gilbert sequencing reactions carried out on the two strands for orientation. Lanes 1 and 5, patterns of DNase I cleavage in absence of proteins. Lanes 2 and 6, 3 and 7, 4 and 8, pattern of DNase I cleavage after binding reaction with the 0.3 M KCl fraction in the presence of 4 ng of DNase I per μ l and 1.8 (lanes 2 and 8), 2.4 (lanes 3 and 9), 3 (lanes 4 and 10), 3.6 (lanes 5 and 11) and 4.2 (lanes 6 and 12) μ g of proteins. (B) Summary of the results shown in panel A. Numbers indicate the position regarding the transcription initiation site (+1).

tained with about 30 molar excess of self-competition for A and 35 molar excess of self-competition for B (Fig. 3B). These results indicate that the binding is specific. The specific complexes obtained with the A or B probes were also competed with by adding molar excess of B and A competitor, respectively (not shown), indicating that the same factor binds to both oligonucleotides.

3.3. DNase I protection

In order to identify this factor, its binding site was delineated by DNase I protection analysis. The factor was partially purified by heparin-agarose chromatography where it was eluted at about 0.3 M KCl. This fraction was then used to carry out the DNase I protection analysis using oligonucleotide B alternately labeled on

the top or the bottom strand. Results are shown in Fig. 4. The binding site of this factor on oligonucleotide B comprises sequence -91 to -77. Comparison of this sequence with oligonucleotide A that also binds this factor, suggests the existence of another binding site at position -125 to -117, the binding consensus being APyNTNCAPyAPyPuTPuCA, where Py is C or T, Pu is A or G, and N is one of the four bases. Protection experiments with the whole LTR (not shown) confirmed this result. Comparison of this 15 nucleotide sequence with the TFD and GNOMIC databases using the SIG-NUC program [24] does not reveal any striking similarity with known nucleic signals.

4. DISCUSSION

Transposable elements are vertically transmitted as classical genetic information from parents to offspring, but horizontal transfer has also been argued [14–17], and even the possibility of interspecies transmission cannot be excluded.

This paper reports the ability of a *Drosophila* retrotransposon promoter to function in a human monocytic-like cell line. Our experiments also describe the detection of at least one proteic factor that specifically interacts with the 1731 LTR in vitro. The DNA binding site, as compared to nucleic signal databases, does not allow the assimilation of this factor to any known transcription factor but it might represent the phorbol ester-activated protein present in U937 cells. Further experiments are needed to test this hypothesis.

This work should be considered as an initial attempt at identifying *trans*-activating factors that interact, in an heterologous system, with a *Drosophila* retrotransposon promoter: it should be placed in an evolutionary context indicating that the use of a heterologous molecular machinery is not a barrier to interspecies transmission of retrotransposons. Furthermore, heterologous transfection appears to be a good experimental system to define the common mechanisms controlling retrovirus and retrotransposon promoter activity and to establish their evolutionary link. Although retrotransposons have not been further demonstrated to be infectious, mostly because of the absence of an env gene, they might be, under some conditions. 1731 comprises, for example, a third open reading frame, just upstream the 3' LTR, the sequence of which does not include any env gene. Translation of a reporter gene when introduced into this third ORF was shown to be possible (C. Coulondre and M. Best-Belpomme, in preparation). On the one hand, this third ORF can be considered as a vestige of an ancestor env gene; but on the other hand, one can imagine that setting up the env gene in frame with this ORF could restore the infectiousness.

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