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RNA polymerase III control elements are required for *trans*-activation by the murine retroviral long terminal repeat sequences

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ABSTRACT

RNA leukemia viruses induce T-cell lymphoblastic lymphomas or myeloid leukemias. Infection of cells with Moloney murine leukemia virus (M-MuLV) up-regulates the expression of a number of cellular genes, including those involved in T-lymphocyte activation. Previously, we demonstrated that this up-regulation occurs via the *trans*-activation activity of the M-MuLV long terminal repeat (LTR) sequences which produce an LTR-encoded transcript. Sequence analysis of the LTR revealed a potential transcription unit for RNA polymerase III (Pol III) within the U3 region that is actively occupied by Pol II factors. Here, we provide the direct evidence of involvement of Pol III in the *trans*-activation process and demonstrate the precise localization of the intragenic control elements for accurate and active Pol III transcription. Deletions of a copy of the directed repeats and further immediate upstream sequences significantly abrogated the generation of LTR-encoded transcript and abolished the *trans*-activational activity, whereas the deletion of a copy of directed repeats alone proportionally reduced the transcript size, but still retained moderately high *trans*-activational activity. In electrophoretic mobility shift assay, the fraction containing a multiple transcription factor TFIIIC complex strongly bound to the LTR-U3 probe containing the essential control elements. The specificity of the DNA-TFIIIC interaction was confirmed by conducting competition assays with DNA fragments containing a genuine Pol III-transcribed gene, VA1, and by vaccinia virus infection which stimulates the expression of Pol III factors. However, a deletion mutant lacking an essential control element bound to the TFIIIC complex poorly, consequently resulting in weak Pol III transcription as assessed by an IRES-GFP reporter system. This correlation strongly supports the possibility that the generation of LTR-encoded transcript is directed by Pol III. Therefore, this finding suggests the involvement of Pol III transcription in the retrovirus-induced activation of cellular genes, potentially contributing to leukemogenesis.

1. Introduction

A number of different types of recombinant retroviruses have been isolated from thymic neoplasms of mice. Moloney murine leukemia virus (M-MuLV) is a C-type non-acutely transforming retrovirus, which lacks oncogenes, but induces lymphoid neoplasia in mice after a long latent period through an indirect mechanism [1]. Recent studies have demonstrated that the genomic long terminal repeat (LTR) sequence determines the leukemogenicity and latency of M-MuLV-induced leukemia [2,3]. The LTR sequence of other MuLVs, including Friend-mink cell forming MuLV causing erythroid leukemia, is also known to determine the pathogenicity and tropism [4]. The LTR sequences contain enhancer elements, in addition to RNA polymerase II (Pol II) promoter elements, in the U3 region. It was previously reported that the M-MuLV LTR profoundly enhances the expression of cellular genes, including those encoding a number of proteins involved in T cell activation [5,6] and the *c-jun* proto-oncogene [7]. It is believed that the tandemly repeated sequences of the LTR are responsible for *trans*-activation, due to their ability to generate the LTR-encoded transcript.

Analysis of the short LTR sequences suggested the involvement of RNA polymerase III (Pol III) transcription in *trans*-activation. A major transcript is believed to be encoded by the putative intragenic promoter sequences of the U3 region. In general, Pol III-transcribed genes are characterized by an intragenic split promoter consisting of two major components, A and B Blocks [8]. Studies on Pol III transcription with relatively large deletions of the putative control elements proposed a set of potential intragenic control elements within the U3 region [9]. RT-PCR and *in vitro* transcription experiments in the presence of Pol II or III inhibitors indirectly supported

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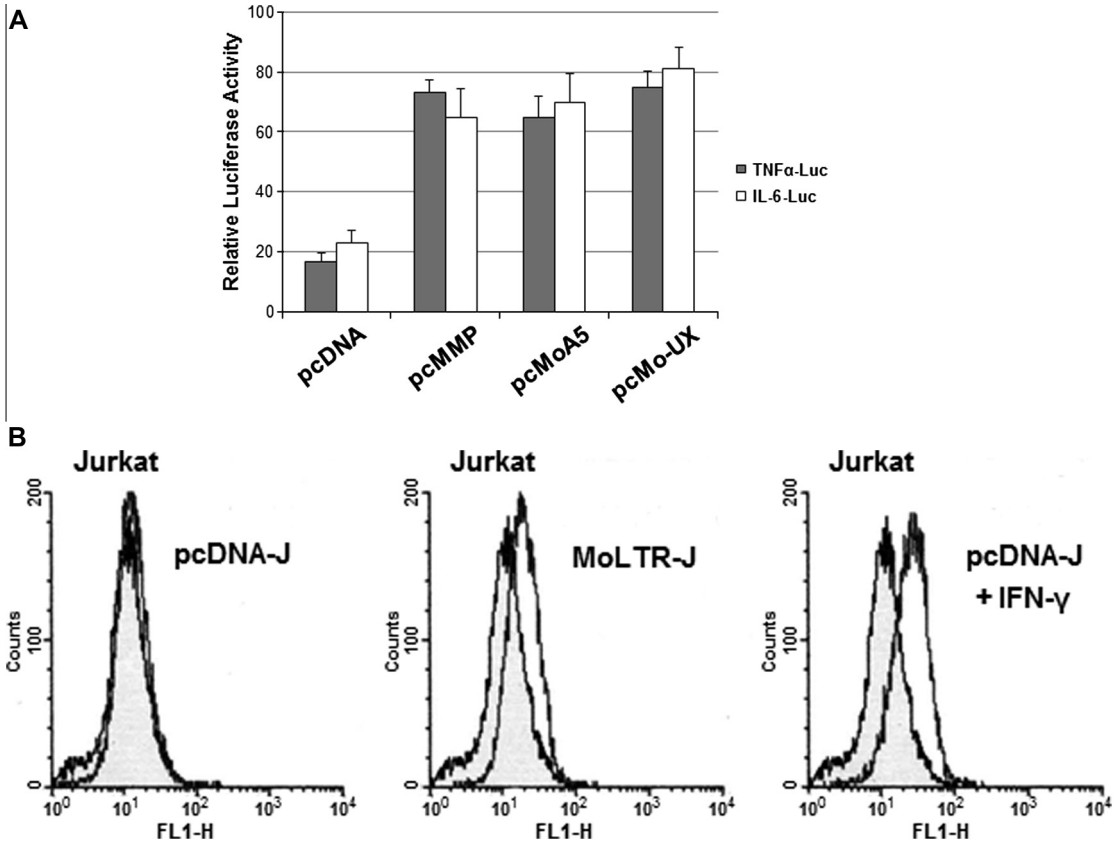

Fig. 1. *Trans*-activation of cellular genes by retroviral LTR sequences. (A) Jurkat cells were co-transfected with a reporter pTNFα-Luc or pIL6-Luc, and the indicated plasmids: pcDNA, pcMMP, pcMoA5, or pcMo-UX, and harvested after 24 h for luciferase assays. The data were expressed as means ± SD for at least three independent experiments. (B) FACS analysis of MHC class I antigen expression was carried out on pcDNA-J and MoLTR-J cells, which were Jurkat cells stably transfected with pcDNA or pcMoA5, respectively, and on Jurkat cells treated with recombinant human IFNγ (100 U/ml) for 48 h. Cells were stained with an antibody against native human MHC class I (W6/32).

the presence of the Pol III transcription unit in the U3 region. However, no direct experimental evidence was provided to ensure the putative Pol III transcription unit within the U3 region is functional in intact cells. In order to provide evidence for the existence of the active Pol III transcription unit and its correlation with functional activity, the localized intragenic control elements need to be identified more precisely, and their capability to interact with an essential Pol III factor, including the common factors TFIIIC and TFIIIB, in cells needs to be determined.

In this study, the functional role of a critical control element of the LTR U3 region in Pol III transcription and *trans*-activation was demonstrated. Direct evidence was provided to support the fact that the generation of the LTR-encoded transcript is closely correlated with the interaction with TFIIIC. This finding indicates an alternative mechanism of retrovirus-induced dysregulation of cellular genes.

2. Materials and methods

2.1. Plasmids

Plasmid pMoV9, a cloned provirus of M-MuLV, was used for LTR expression and construction of other retroviral vectors [10]. Plasmid pcMoA5 is a pMoV9 derivative containing a single 5′ LTR genomic sequence in pcDNA. The 3′ deletion mutant pcMo-UX was a pcMoA5 derivative with deletion downstream from the *XbaI* site. pcMo-UXdV is identical to pcMo-UX except for a deletion of *EcoRV-EcoRV* in the U3 region. pcMo-UXPdV was the same construct as pcMo-UXdV, but with total deletion of 86 bp upstream from the 5′ end of the *EcoRV* site using a recombinant circle PCR mutagenesis reaction. A retroviral transfer vector pcMMP containing both the 5′ and 3′ LTRs of the provirus was provided by Dr. R.C. Mulligan. A reporter plasmid, pTNFα-Luc, containing TNFα gene promoter (−1274) driving the luciferase gene, was a gift from Dr. B.P. Giroir. Plasmids pG2A-MoUX and pG2A-MoUXPdV were constructed by inserting the LTR portion of pcMo-UX and pcMo-UXdV into the pGEM2 vector, respectively. Plasmids pG2A-MoUX-IRES-GFP and pG2A-MoUXPdV-IRES-GFP were constructed by inserting a 1372 bp fragment of the enhanced GFP-encoding region under the control of the internal ribosome entry site (IRES) into the *XbaI* sites of the pG2A-MoUX and pG2A-MoUXPdV, respectively.

2.2. Cell culture and virus infection

Balb/c 3T3, human osteosarcoma 143B and human T lymphoblastoid Jurkat cell lines were obtained from the American Type Culture Collection (Rockville, M.D., USA) and maintained as recommended. Transfection was conducted using Effectene reagent (Qiagen). Co-transfection with the pRSV-β-galactosidase plasmid and normalization of β-galactosidase activity were carried out to assess transfection efficiency. Long-term cell lines pcDNA-J and MoLTR-J were generated by co-transfection of Jurkat cells with the selection marker pSV2neo and pcDNA or pcMo-UX, respectively, and subsequently selected in G418-containing medium.

Recombinant vaccinia virus harboring the T7 polymerase gene (vTF7-3) was a kind gift of Drs. T. Fuerst and B. Moss (Division of AIDS, NIAID, NIH, USA). A monolayer of 143B cells was treated with VTF7 at a multiplicity of infection of 2 to ensure infection of >95% of the cells [11].