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Nuclear factor-1 (NF-1) binds to multiple sites within the transcriptional enhancer of Moloney Murine Leukemia virus

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The transcriptional enhancer of the Moloney Murine Leukemia virus (Mo-MuLV) is comprised of a 75-bp direct repeat, each of which contains multiple binding sites for transcription factors. The occupancy of these sites determines the tissue specificity of expression and disease tropism of the virus. The identification of proteins that bind to this enhancer is therefore required in order to understand the molecular basis of this viral–host interaction. Analysis of the nucleic acid sequence of the Mo-MuLV has identified 4 potential binding sites for the transcription factor NF-1. Evidence is presented using DNase I protection analysis that NF-1 binds to these 4 sites within the enhancer. The potential role of NF-1 binding in tissue specific expression of Mo-MuLV is discussed.

Gene expression; Transcription factor; DNA-binding protein; DNase I protection

1. INTRODUCTION

The Moloney Murine leukemia virus (Mo-MuLV) contains a transcriptional enhancer within the U3 region of the long terminal repeat (LTR) that is comprised of a 75-bp repeated sequence located approximately 160 bp 5′ to the initiation site of transcription [1]. This transcriptional enhancer controls the tissue specific expression of the LTR [1,2,3,4] and contains the primary genetic determinants of the leukemogenicity and disease specificity of the virus [5,6].

Like other viral and cellular transcriptional enhancers, the Mo-MuLV enhancer is composed of multiple binding sites for cellular transcription factors [7,8]. The array of transcription factors that bind to these sequences in different cell types presumably regulates the activity of the LTR and thus the oncogenic potential of the virus [6,9]. The identification and characterization of these subunits of the viral enhancer and the cellular factors that interact with them are therefore essential towards generating an understanding of the basis of this virus–host interaction.

Previous studies have identified binding sites for at least 6 cellular factors within each 75-bp element of the Mo-MuLV enhancer. These include binding sites for leukemia virus factors a, b, and c (LVa, LVb and LVc), the CCAAT/enhancer-binding protein (C/EBP), the glucocorticoid response element (GRE) as well as potential binding sites for the transcription factor nuclear factor-1 (NF-1) [7]. With respect to NF-1 binding, each copy of 75-bp repeat of the Mo-MuLV enhancer contains two non-identical copies of sequences highly homologous to the consensus NF-1 binding site (5′-TGGA/C N_5GCCAA [10]). These potential NF-1 sites have been highly conserved among murine leukemia viruses [11,12] and thus are likely to be functionally important for expression of viral genes. While mutations in these sites have been shown to decrease the activity of the enhancer [9], direct binding of NF-1 to the LTR has not been demonstrated. Here I present biochemical evidence showing that these NF-1 sites specifically bind protein present in HeLa cell nuclear extracts. Furthermore, affinity-purified NF-1 derived from HeLa cells binds to the same 4 sites in the enhancer demonstrating directly that NF-1 binds to the Mo-MuLV transcriptional enhancer.

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2. EXPERIMENTAL

Nuclear extracts of HeLa cells were prepared essentially by the method of Dignam et al. [13]. The crude nuclear extract was subjected to heparin-agarose chromatography and fractions eluting between 0.15 M and 0.4 M KCl were collected and used for binding reactions. HeLa heparin-agarose fractions were kindly provided by Dr J. Nyborg (Colorado State University, Ft. Collins). NF-1 was purified from HeLa nuclear extracts by DNA-affinity chromatography [14] and was a gift from Dr R. Markowitz (University of Colorado, Boulder).

A 470-bp *Cla*I–*Xba*I restriction fragment (positions 7676–8116) [15] from the U3 region of the Mo-MuLV LTR was subcloned into puc19 at the *Sma*I site of the polylinker. The fragment was released from the vector by digestion with *Eco*RI and *Hin*dIII. The DNA probe was 5′-end labeled on either the non-coding or coding strands by reaction with [γ-^{32}P]ATP and polynucleotide kinase or by reaction with [α-^{32}P]dATP followed by digestion at the *Bam*HI site of the polylinker to release ^{32}P incorporated at one end of the molecule. Binding reactions for DNase I protection analysis contained 10 fmol ^{32}P-labeled DNA, 1 μg poly(dI-dC), 25 mM Tris-HCl (pH 7.5), 0.5 M EDTA, 1 mM DTT, 50 mM KCl, 6.25 mM $MgCl_2$ and 10% glycerol in

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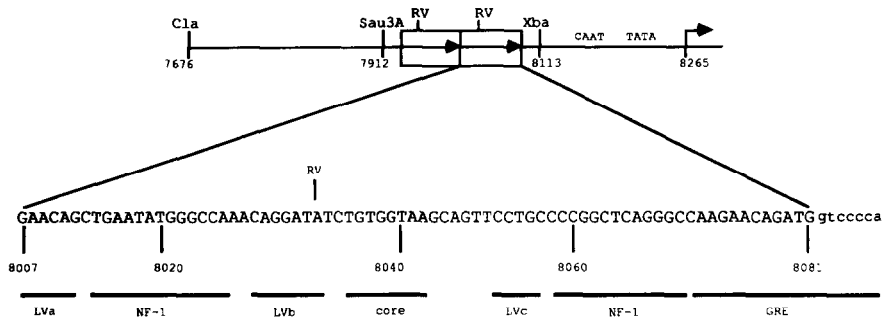
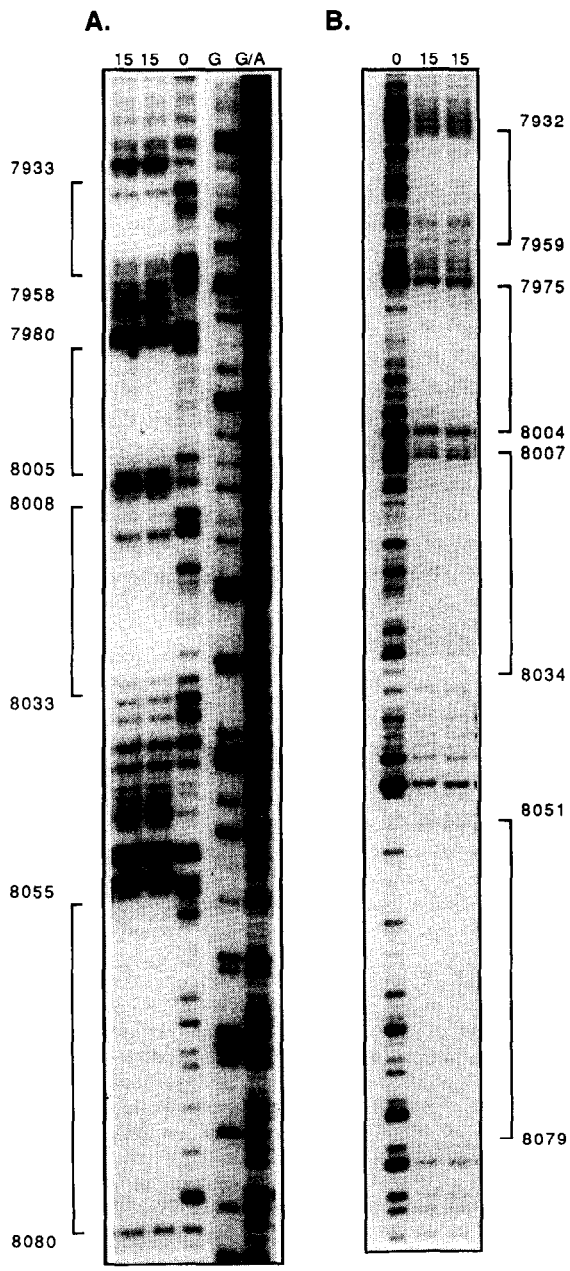


Fig. 1. Organization of the U3 region of the Mo-MuLV LTR. Sequences are from the 3′ end of a Mo-MuLV provirus extending from the *Cla*I site (7676) in the *env* gene to the start site of transcription (arrow at 8265) [15]. The TATA and CAAT boxes lie upstream to the transcription start site. The transcriptional enhancer is comprised of a 75-bp perfect direct repeat (boxed arrows). The nucleotide sequence of one copy of the direct repeat is shown. The reported binding sites for nuclear factors [7] are underlined.



a final reaction volume of 50 μl. Binding reactions were carried out on ice for 15 min, treated with DNase I (0.2 to 2 μg/ml), extracted with phenol/$CHCl_3$ 1:1 and electrophoresed in 7% acrylamide/8 M urea sequencing gels. Maxam-Gilbert sequencing reactions were run in adjacent lanes for DNA sequence positions.

3. RESULTS AND DISCUSSION

The organization of the U3 region of the Mo-MuLV LTR is shown in Fig. 1. The predicted NF-1 binding sites [7] map to the 75-bp direct repeat and occur at identical positions within each repeat. NF-1 site 'a' maps to positions 7939–7952 and 8014–8027 and NF-1 site 'b' maps to positions 7984–7997 and 8059–8072. To determine whether these sites interact with nuclear proteins, DNA containing the 75-bp repeat (between positions 7676 and 8116) was end-labeled with ^{32}P at position 8116 and analyzed by DNase I protection analysis using heparin-agarose chromatography fractions of nuclear extracts from HeLa cells. As shown in Fig. 2, two regions of each copy of the 75-bp repeat are protected from DNase I digestion indicating that nuclear proteins are binding to these sites. The four protected regions span positions 7933–7958, 7980–8005, 8008–8033 and 8055–8080. Each of these regions contains within it the predicted NF-1 binding site.

Since the regions of protection are quite large (~25 bp) and contain within them other potential binding sites, nuclear DNA binding proteins other than NF-1 could be responsible for this pattern of protection over the 75-bp repeat. This question was addressed by assaying for NF-1 binding using purified NF-1 in the DNase

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Fig. 2. DNase I protection analysis of the Mo-MuLV transcriptional enhancer. Binding reactions were carried out with a 470-bp *Cla*I-*Xba*I fragment that was ^{32}P-labeled at either the coding (A) or non-coding (B) strand 35 bp 3′ to the enhancer at the *Xba*I site (see Fig. 1). The DNA was incubated in the absence (0) or presence (15 μg) of heparin-agarose enriched HeLa nuclear extract. Regions of protection are bracketed with the numbers of the corresponding nucleotide sequence. The positions of the sequences were determined by Maxam-Gilbert sequencing reactions.

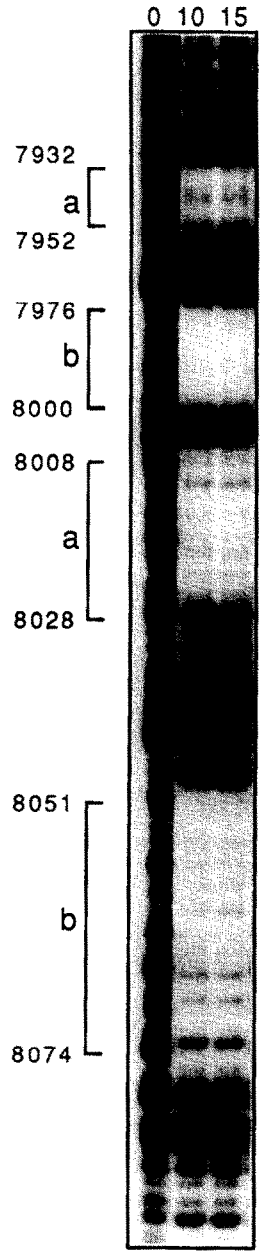

Fig. 3. DNase I protection analysis of the Mo-MuLV transcriptional enhancer after incubation with affinity purified NF-1 (10 and 15 μl, corresponding to 0.3 and 0.45 μg of protein, respectively). Control lanes (0) contained no added protein. Protected regions on the non-coding strand are indicated by brackets along with the numbers of the corresponding nucleotide sequence. 'a' and 'b' refer to the putative NF-1 binding sites first identified by Speck and Baltimore [7].

I protection assay. As demonstrated in Fig. 3, purified NF-1 was found to bind within the 75-bp repeat to the same 4 sites observed with Hela cell extracts. The NF-1 sites, labeled 'a' and 'b' occur at positions 7932–7952, 8007–8028 and 7976–8000, 8051–8074, respectively. All these sites show a high degree of homology to the NF-1 consensus binding site [7,10].

In summary, the data presented here demonstrate that NF-1 binds to 4 positions within the transcriptional enhancer of Mo-MuLV. This result together with previous results showing that mutations in either the NF-1b or both the NF-1a and b binding sites lead to a preferential decrease in promoter activity in fibroblasts but not lymphoid cells [9] indicate that NF-1 binding may play a role in cell type specific expression of the LTR. NF-1 has been shown to comprise of a family of polypeptides that are active in transcriptional activation which are encoded by multiple species of mRNAs [16,17]. It has been proposed that different cell types may encode different subsets of NF-1 having altered activities or binding specificities [17]. Thus the array of NF-1 proteins in the nucleus could potentially alter the expression of the LTR. In addition, the presence of other nuclear factors that bind to nearby or overlapping sites and thus compete with NF-1 for binding may also play an important role in regulating expression from the LTR. Further studies will determine the relationship of cell type specific NF-1 species having varied activities or affinities for different binding sites in the enhancer to the cell type specific expression and disease tropism of Mo-MuLV.

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