Identification of a Nuclear Matrix-associated Region of the c-myc Protooncogene and Its Recognition by a Nuclear Protein in the Human Leukemia HL-60 Cell Line

Robin H. Chou, Judy R. Churchill, Marcella M. Flubacher, Diane E. Mapstone, and JoLynda Jones

Department of Anatomy [R. H. C., J. R. C.] and Program of Molecular Biology and Biotechnology [R. H. C., M. M. F., D. E. M.], Hahnemann University, Philadelphia, Pennsylvania 19102

ABSTRACT

A nuclear matrix (NM)-associated region (MAR) of the protooncogene c-myc is identified in a human leukemia cell line (HL-60). A binding assay between isolated NM and 32P-end-labeled c-myc fragments in the presence of unlabeled competitors was used, and a 3′-end DraI/DraI fragment of 172 base pairs containing the first of the two polyadenylation [poly(A)] signals was identified as an in vitro MAR. Direct detection of endogenous c-myc fragments remaining NM bound after restriction digestion was used, and an in vitro MAR has been identified as the Clal/EcoRI 1.4-kilobase pair fragment containing the 172-base pair in vitro MAR fragment. In addition, a nuclear protein (M, = 25,000, p25) demonstrating preferential binding to the 172-base pair c-myc MAR has been identified and partially purified. This protein is diminished in the nuclei of the cells induced by phorbol ester to undergo macrophage differentiation. Footprint analysis shows that p25 binds to two regions of the 172-base pair fragment. One contains the first of two poly(A) addition signals and a topo II box-like sequence, and the other (AATTTCATCCTAGTA) is 17 base pairs downstream of the first poly(A) signal.

INTRODUCTION

The nuclear matrix can be isolated by treating nuclei with nuclease digestion and high salt extraction (1). This nuclear framework is responsible for anchoring topological domains or loops of interphase chromatin (2–4). Some of the loop attachment regions have been reported to be enriched for sequences related to the consensus sequence of the DNA topoisomerase II cleavage site (5–9). The MARs have also been associated with cis-acting sequences related to transcriptional regulation (6). The MAR of the mouse c- and heavy chain immunoglobulin was found to be associated with tissue-specific transcriptional enhancers (10). In addition, the 5′-MAR of the Drosophila alcohol dehydrogenase, Sgs-4, and fushi tarazu genes were localized in the area containing sequences required for the induction of high transcriptional activity during development (5, 7, 11).

A great deal of information regarding transcriptional regulation of the protooncogene c-myc has recently been evolved. Activation of c-myc expression can be attributed to various mechanisms including gene amplification (12, 13), insertion of viral transcription regulatory elements near the gene (14), chromosomal translocations (15), and removal of a negative DNA enhancer element normally associated in cis with the gene (16). A set of trans-acting factors has been suggested to provide a means for modulating the function of the negative enhancer (17). The existence of protein factors regulating c-myc control elements has been suggested since identification of DNase I hypersensitive sites in c-myc chromatin can be correlated to the transcriptional state of the gene (18–22). Nevertheless, the MAR of the c-myc gene are yet to be identified.

HL-60 cells can be induced to undergo macrophage differentiation by agents such as phorbol esters (23) and granulocytic differentiation by agents such as retinoic acid (24). Numerous phenomena occur during differentiation of HL-60 cells. They include activation of membrane ion channels (25), changes of nucleosomal components (26), and changes of chromatin conformation at c-myc and c-fos gene loci (27). However, the high level of c-myc gene transcription expressed in HL-60 cells prior to differentiation and the repression of these genes after differentiation (28) make this cell line a useful tool for identifying c-myc MAR and probing its role in regulating c-myc expression during leukemic cell differentiation. Here we report the identification of a region containing the 3′-end sequence of exon 3 and a polyadenylation signal as a c-myc MAR and a HL-60 nuclear protein which binds to the c-myc MAR with preferential affinity.

MATERIALS AND METHODS

Cell Cultures. The human promyelocytic leukemia cell line, HL-60, was maintained under conditions described elsewhere (27). Approximately 2 x 10⁶ non-treated HL-60 cells and cells treated with 500 nM PMA (Sigma Chemical Co., St. Louis, MO) for 48 h at a density of 4 × 10⁶ cells/ml were harvested for isolation of nuclear proteins. PMA-treated cells were harvested by scraping with a rubber policeman.

5RE-myc Probes. pHAS-1 plasmid (11) containing a 8.07-kbp human c-myc insert was purchased from American Type Culture Collection. The plasmids were digested with HindIII/Clal/EcoRV/XhoI/EcoRI. After restriction digestion the DNA fragments were separately isolated from preparative agarose gels (29) and recombined on an equimolar basis. Each recombined set of fragments was dephosphorylated and 5′-end labeled with [γ-32P]ATP using T4 polynucleotide kinase (29). Subfragments of the 1.4 kbp-fragment were generated by digestion with DraI of gel-purified 1.4-kbp fragment (Fig. 1). This set of subfragments was dephosphorylated and 5′-phosphorylated as described above.

In Vitro Identification of c-myc MAR. Assay of DNA binding to nuclear matrices was performed as previously described by Cockerill and Garrard (6). In brief, nuclei from either control or PMA-induced HL-60 cells were isolated by Dounce homogenization and separated from cytoplasm through 2.0 M sucrose. Nuclear matrices were prepared by DNase I digestion (100 μg/ml for 2 h) in 0.25 M sucrose in the presence of 3 mM MgCl₂ and 1 mM CaCl₂, followed by three extractions with 2.0 M NaCl-10 mM Tris-HCl, pH 7.4, 4–0.5 mM PMSE-0.25 mg/ml bovine serum albumen. DNA fragments labeled at 5′-ends (2.0–20.0 ng containing 1.5 × 10⁸ to 1.2 × 10⁹ cpm) were incubated with 1.5 × 10⁶ nuclear matrices in the presence of salmon testis DNA as competitor (2.0–10.0 μg) in assay buffer (50.0 mM NaCl-10.0 mM Tris-HCl,
A PROTEIN BINDS NUCLEAR MATRIX-ASSOCIATED REGION OF c-myc

A 4-kbp fragment containing the 1.4-kbp c-myc insert (Fig. 1, designated as SRE-mjr) was used as probes for Southern blotting. The DNA was digested with HindIII/Clal/EcoRI and fractionated by agarose gel electrophoresis (31). Purification of protein p25. Purification of protein p25 by gel elution was accomplished by electrophoresis of 1.5 mg of the crude 0.35 M NaCl extract of HL-60 cells into a 15% preparative SDS-PAGE for 5 h at 25 mA. The region of protein p25 was excised without staining, soaked in 4 mM urea in transfer buffer to remove SDS, and ground into small pieces, and proteins were eluted by using an electrophoretic apparatus (BioRad, Richmond, CA). An aliquot of the concentrated eluate was then analyzed by a 15% SDS-PAGE as shown in Fig. 7, lane 4. Protein quantitation was done by the method of Bradford (36) using reagents purchased from BioRad with γ-globulin as standard.

Identification of p25-Binding Domains on the 1.4-kbp Fragment. In order to identify the DNA region(s) responsible for specific protein binding within the 1.4-kbp, 3' Clal/EcoRI c-myc DNA fragment, purified p25 was incubated with a mixture of 5'-end-labeled 764-, 464-, and 172-base pair DNA subfragments obtained by DraI digestion (Fig. 1) and analyzed by electrophoretic gel retardation assay (37). The incubation volume of 25 μl contained 15.2 mM Tris-HCl (pH 7.5), 8 mM MgCl2, 2.24 mM EDTA, 1.16 mM mercaptoethanol, 76 mM NaCl, 0.008% bromophenol blue, and 3.2% glycerol. Protein solution was added last to binding mixtures containing 0.2 ng 5'-end-labeled DNA fragments (106 cpm) and indicated amounts of competitors to initiate a 30-min incubation at 20°C. The base composition of DNA sequences responsible for binding of protein p25 to fragments of 172 and 464 base pairs were evaluated by analyzing binding activity in the presence of unlabeled poly[d(A-T)], poly[d(I-C)], poly[d(G-C)], and HaeIII-digested αX174 DNA fragments as competitors in the electrophoretic retardation assay as described earlier. In the binding reactions, 0.2 ng of labeled probe mixture of 764-, 464-, and 172-base pair fragments containing 104 cpm was incubated with 13 μl of purified protein p25 (0.1 mg/ml) with or without the presence of 5 ng 5-μg of the competitors. Separation of protein-bound from free DNA fragments was via diffusion blotting at room temperature for 36 h in transfer buffer (10 mM Tris-HCl, pH 7.5-2 mM Na2EDTA-0.5 mM mercaptoethanol-0.14 and 0.35 M NaCl in buffer (10 mM Tris-HCl, pH 7.4-1 mM MgCl2-0.1 mM NaEDTA-1 mM MgAc-50 mM NaCl) to allow DNA binding. The binding reaction was terminated by washing the nitrocellulose strip in the presence of 2000-fold weight excess, protein-bound DNA fragments were ethanol precipitated and further analyzed by electrophoresis through 1.5% agarose gels in TPE buffer (29). The gels were dried and autoradiographed.
A PROTEIN Binds Nuclear Matrix-AssOCIATED REGION of c-myc

low-ionic strength (LIS) (3.3 mM sodium acetate-6.7 mM Tris-HCl-1 mM EDTA, pH 7.5) gel electrophoresis (3.88% acrylamide, 0.12% bisacrylamide, 0.1% ammonium persulfate, 0.1% N,N',N'-tetramethylethylenediamine in LIS). LIS gels were preelectrophoresed at 100 V for 1 h prior to gel retardation assay. Samples were run at 200 V for 2.5 h at room temperature with buffer recirculation. Following electrophoresis, gels were dried on filter paper and autoradiographed.

Another electrophoretic retardation assay was done using a purified 172-base pair fragment of c-myc and a 121-base pair Dral/Dral fragment from the first intron of c-myc as binding substrates of the partially purified p25. Synthetic poly[d(G-C)] was used as competitor DNA.

DNase I-Footprinting Assay. The 172-base pair Dral/Dral c-myc fragments were subcloned into the Smal site of the pUC-18 polylinker (Boehringer/Mannheim, Indianapolis, IN) as pUC-172 plasmid. The pUC-172 was isolated and purified from host Escherichia coli and linearized by EcoRI digestion, dephosphorylated, phosphorylated at 5'-end with T4 polynucleotide kinase using [γ-³²P]ATP, and digested with BamHI to generate a singly end-labeled 194-base pair fragment containing the 172-base pair Dral/Dral c-myc insert. DNase I footprinting was done as described (38). In a reaction volume of 25 μl, 1.2 ng of 194-base pair probe was incubated with partially purified p25 in the presence of 500 ng of polyd(G-C) as competitor. After a 30-min incubation at room temperature, 50 μl of stop buffer (200 mM NaCl-20 mM EDTA-1% SDS-250 μg/ml tRNA) was added. Samples were phenol/chloroform extracted, ethanol precipitated, and analyzed on 8% polyacrylamide-8 M urea sequencing gel (29). Chemical cleavage and sequencing of G and A bases was performed as described (29).

RESULTS AND DISCUSSION

In Vitro Identification of the c-myc MAR. During interphase of the cell cycle, the nuclear DNA is organized into supercoiled loop domains of about 50–100 kbp which may be attached to the nuclear matrix via topoisomerase II cleavage sites and A+T-rich sequences (6-7). In vitro assay of c-myc DNA binding to HL-60 NM was performed by incubating labeled, cloned c-myc subfragments (5RE-myc, Fig. 1) as binding probes with isolated NM structures. The NM structures were then isolated together with the NM-bound DNA fragments by centrifugation. These fragments were then purified and analyzed as described in “Materials and Methods” and shown in Scheme 1. As shown in Fig. 2, lane 3, incubation of six labeled-fragments of 5RE-myc probe mixture with HL-60 NM without the presence of competitor DNA resulted in preferential binding of the 1.4-kbp fragment (arrow) to the NM structures. The control pattern of the 5RE-myc bands is shown in lane 2. When the same set of

Fig. 3. Autoradiograms of retention of subfragments of c-myc 1.4-kbp fragments by NM. A mixture of ³²P-labeled restriction fragments obtained by Dral digestion of 1.4-kbp c-myc fragment were incubated with NM obtained from 1.5 x 10⁶ nuclei of noninduced (A) and PMA-induced HL-60 cells (B), respectively. The NM-retained DNA fragments were isolated by centrifugation and analyzed by electrophoresis and autoradiography as described in “Materials and Methods.” Std., restriction fragments of HindIII-digested ϕX174 DNA; lane 1, NM-bound subfragments of 1.4-kbp fragments without the presence of nonlabeled ST DNA; lane 2, with the presence of 2 μg (1000-fold) ST DNA; lane 3, with the presence of 10 μg (5000-fold) ST DNA; arrow, the 172-base pair fragment.
A PROTEIN BINDS NUCLEAR MATRIX-ASSOCIATED REGION OF c-myc

The region homologous to the detected in vitro c-myc MAR, in vivo MAR identification experiments were performed on HL-60 cells as described in “Materials and Methods.” The isolated nuclei were extracted with 2.0 M NaCl, and the residual structures (halo nuclei) were digested with Clal and EcoRI. The NM-associated DNA fragments were then isolated from enzyme-released fragments by centrifugation and Southern hybridized to the labeled 1.4-kbp c-myc fragment (Fig. 1) as probe for the in vivo MAR sequences (Scheme 1). As shown in Fig. 4, a discrete 1.4-kbp band was detected in the NM-associated DNAs (lane 2) but not in enzyme-released DNAs (lane 3). These results suggest that the genomic c-myc region spanning between Clal and EcoRI sites contains an endogenous c-myc MAR sequences. These results, however, do not exclude the existence of other endogenous c-myc MARs. It would not be surprising that other c-myc sequences are also able to bind to the matrix, although their binding affinity may not be comparable to the affinity of the 1.4-kbp region.

Identification of c-myc MAR-Binding Protein. Nuclear proteins isolated from HL-60 cells were analyzed for their DNA-binding activities by using Southern-Western protein-DNA binding analysis. The 0.14–0.35 M NaCl-extracted nuclear proteins were separated by SDS-PAGE and blotted to a twin set of nitrocellulose papers by diffusion (34, 35). Amido black-stained patterns of proteins blotted on the nitrocellulose are shown in Fig. 5A. Two HMG proteins (39), HMG 1 and HMG 2, and a protein band with a relative molecular weight of 25,000 (designated as protein p25) were resolved as the major protein components (A, HL-60 lane). A decrease of protein p25 and an increase of p52 and p25 were noticed after PMA induction (Fig. 5B). These bands were detected by autoradiograms obtained after incubation of nitrocellulose paper-blotted proteins with c-myc probes in the presence of unlabeled salmon testes DNA in 2000-fold excess amount.

In Vivo Identification of the c-myc MAR. To confirm whether genomic c-myc sequences are anchored to the NM structure at

---

Fig. 4. Identification of in vivo MAR of c-myc. Southern hybridization of the Clal/EcoRI-released (SN) and MAR halo nuclear DNA fragments separated by agarose gel electrophoresis to the 1.4-kbp Clal/EcoRI fragment of the pHSR-1 as probe. Std., DNA size standards.

Fig. 5. Identification of the protein p25 and its binding to 32P-labeled SRE-myc fragments. A. Amido black-stained patterns of proteins blotted onto nitrocellulose paper. Lane 1, molecular weight standards (Std.); lanes 2 and 3, nuclear extracts from noninduced (HL-60) and induced (PMA) HL-60 cells. B, autoradiograms obtained after incubation of nitrocellulose paper-blotted proteins with c-myc probes in the presence of unlabeled salmon testes DNA in 2000-fold excess amount. Ordinate (k), M, in thousands.

---

Downloaded from cancerres.aacrjournals.org on April 20, 2017. © 1990 American Association for Cancer Research.
A PROTEIN BINDS NUCLEAR MATRIX-ASSOCIATED REGION OF c-myc

The sequence-specific binding activity of p25 was then further tested by gel retardation assay (37) using the three subfragments of the 1.4-kbp fragments (Fig. 1) as labeled binding substrates. As shown in Fig. 8, upward titration of protein p25 increases retardation of the 172-base pair fragment. In A, lane 1, the banding pattern of the three probes is shown in the absence of protein p25. Lanes 2–5 show the patterns of probes after incubation with 2.5, 5, 9, and 18 μl of protein p25 (0.1 mg/ml). A retarded band of the 172-base pair fragment becomes apparent after incubation with 5 μl of protein, and the intensities of retarded bands are protein dose dependent (lanes 2–5). At a level of 18 μl of purified p25, a cascade of retarded 172-base pair fragments was observed as well as a retarded band of the 464-base pair fragment (lane 5). No retardation of 764 base pairs is detected. Lack of a retarded band of the 764-base pair probe is not due solely to too large a fragment size, since in other experiments retardation of similar large fragments can be demonstrated under the same gel conditions (data not shown).

The competitive binding assays were then performed by using φX174, poly[d(I-C)], poly[d(G-C)], or poly[d(A-T)] as competitors. As shown in B–E, the poly[d(A-T)] appeared as the most potent competitor. It took 500 ng of poly[d(G-C)] (D, lane 4), 50 ng of φX174 DNA (B, lane 3), more than 5 ng of poly[d(I-C)] (C, lane 2), or less than 5 ng of poly[d(A-T)] (E, lane 2) to abolish the retarded 172-base pair band. These data suggest that p25 recognizes two binding domains in the 1.4-kbp fragment, and the primary binding domain is located in the A + T-rich region of the 172-base pair fragment. These results are later confirmed by DNase I-footprinting analysis.

An additional control experiment was performed by using a 1 2 3 4 5 6 7 increase of HMG 1 was detected in the extract from PMA-induced cells (A, PMA-HL-60 lane). The sister nitrocellulose protein blot was then incubated with the 5RE-myc probe mixture in the presence of an excess amount of unlabeled salmon testis DNA as described in “Materials and Methods.” As revealed in Fig. 5B, p25 and the HMGs were detected as the major DNA-binding proteins. Each radioactive DNA probe-bound protein band was then excised, and the protein-bound 5RE-myc fragments were dissociated and analyzed by gel electrophoresis as shown in Fig. 6. When compared to the control pattern of the 5RE-myc fragments as shown in lane 2, the HMGs from either noninduced or PMA-induced HL-60 cells showed no significant preferential binding to any particular fragment of the 5RE-myc (lanes 4–7). However, protein p25 retained significantly more of the 1.4-kbp fragment than the other fragments in the 5RE-myc pool (lane 3). Densitometry scanning showed p25 retained the 1.4-kbp fragments 4-fold more than other fragments (data not shown). This degree of difference was not detected with HMGs. These results provide a clue that p25 may preferentially recognize the c-myc MAR.

To verify this possibility, protein p25 was partially purified from the nuclear extract by the gel elution technique as described in “Materials and Methods” and shown in Fig. 7. While p25 was first isolated by 0.14–0.35 M NaCl nuclear extraction as shown in lane 2, extraction of p25 with 5% PCA was more effective (lane 3). p25 was partially purified from the PCA extract and analyzed by electrophoresis on a 15% SDS-PAGE (33) (lane 4).

![Fig. 6. Agarose gel autoradiogram of protein-bound DNA fragments dissociated from nitrocellulose paper-blotted proteins. Lane 1, standard (Std.) DNA sizes of labeled HindIII-digested λ-DNA; lane 2, 5RE-myc fragments; lanes 3–7, DNA fragments dissociated from individual protein bands. Arrows, original point of migration.](https://example.com/f6.png)

![Fig. 7. SDS-PAGE analysis of the partially purified protein p25. Lane 1, protein standards with known Mr values; lane 2, Coomassie blue-stained pattern of the 0.14–0.35 M NaCl nuclear extract from HL-60 cells; lane 3, nuclear proteins extracted with 5% PCA; lane 4, the protein p25 partially purified from the PCA extract.](https://example.com/f7.png)

Downloaded from cancerres.aacrjournals.org on April 20, 2017. © 1990 American Association for Cancer Research.
DNA probe with size similar to the 172-base pair probe and a protein with similar size to p25 in the electrophoretic gel retardation assay. The control probe is a 121-base pair oral/oral fragment from the first intron of c-myc. The control protein is p22, a protein with a molecular weight of 22,000, which was also isolated from the crude extract by electroelution. As shown in Fig. 9, binding reaction with p25, but not with p22, resulted in retardation of the 172-base pair probe (compare lanes 2–4 with lanes 5–7). On the other hand, neither p25 nor p22 retard the 121-base pair probe (compare lanes 2–4 with lanes 5–7). Positive binding control was done by using crude extract as the source of binding proteins (lane 15). The binding assays were performed with an upward titration of φX174 DNA as competitor as described in the legend to Fig. 9.

Sequence Analysis of the c-myc MAR. Both the 172- and 464-base pair fragments contain a polyadenylation signal, AA-TAAA. In addition, the 172-base pair fragment bears two runs of 12-base pair-long A + T regions, and the 464-base pair fragment bears only one run according to the published sequences (40, 41). The 764-base pair fragment contains one 10-base pair-long A + T region. However, only the 172-base pair fragment was detected as the in vitro c-myc MAR-bearing fragment. The 172-base pair fragment is composed of 78 base pairs at the 3'-end of exon 3 and 96 base pairs of flanking sequence. These results are in agreement with the reports that A + T-rich DNA sequences may act as nuclear matrix (6) or scaffold attachment regions (7, 11). The 172-base pair sequence was further analyzed for similarity to the T box or A box sequences found in several Drosophila gene attachment regions (7) as well as for potential topoisomerase II consensus sequences (8). A perfect match to the 15-base pair-long topoisomerase II consensus sequence [topo II box: GTN(A/T)A(T/C)ATTNATNN(G/A)] (8) is not found, although the 172-base pair fragment bears four potential topo II boxes with agreement in 9 of 15 and 12 of 15 bases in the coding strand and 12 of 15 and 13 of 15 in the noncoding strand. It also bears one 10-base pair-long T box, also in the coding strand. Fragment 464 bears neither T nor A boxes. It has one potential topo II box in the noncoding strand. The 764-base pair fragment has a T box and one potential topo II box in the noncoding strand.

DNase I-footprinting analysis (Fig. 10) has revealed two footprints of p25 on the 172-base pair fragment. The upstream
The upstream footprint also includes part of a noncoding strand top II box partial match [8 nucleotides 5' to the coding strand poly(A) addition signal]. This fact also raises a possibility that association of the 172-base pair probe to nuclear matrix structure might be modified by the binding of p25.

Characterization of the Protein. The fact that the protein p25 can be extracted with either 0.35 M NaCl or 5% PCA raised a possibility that the protein p25 is one of the known HMG proteins. Although its significance is not clear at this stage, the drastic diminution of p25 in the nuclei of cells differentiated into macrophages is interesting. Loss of HMG 1 and 2 has been reported in terminally differentiated mouse neuroblastoma and Friend erythroleukemia cells induced by dimethyl sulfoxide (46). The HMG 1 and HMG 2 have greater than 85% similarity in amino acid sequence. Their sizes (approximately 28,000) (47) are larger than protein p25, however, HMG 2 is also shown to be dramatically depleted in organs with decreased proliferative activity (48). In contrast, other investigators have found no correlation between cell proliferation and the abundance of HMG 1 and HMG 2 (49). In our study, HMG 1 actually increased in differentiated HL-60 cells induced by phorbol ester (Fig. 5A). On the other hand, mouse HMG I was reported recently to interact with the 3'-end A + T-rich sequences of genes in vitro (50). The HMG I migrated at the Mr 20,000 region, although its actual molecular weight is only approximately 10,000 (51). The fact that the protein p25 migrated significantly slower than the HMG I and faster than HMG 1 and HMG 2 suggests that the protein p25 is unique. Although the possibility that p25 is a degraded product of HMGs cannot be ruled out, such degradation has to be sequence specific due to the discrete banding pattern of p25 in the SDS-PAGE gel. The fact that poly[d(C-I-C)] cannot compete with the binding of the 172-base pair fragment to p25 as effectively as poly[d(A-T)] suggests that the 2-amino group of guanine in the minor groove is not the only reason preventing high affinity binding of p25 to poly[d(G-C)] sequences. This is a distinct character of p25 when compared to the α-protein (52) or HMG-I.

To date, the signals required for proper 3'-end processing and polyadenylation have been difficult to assess. The highly conserved hexanucleotide AATAAA located upstream of most eukaryotic polyadenylation sites is not in itself sufficient to define 3'-end sequences since this hexanucleotide may exist in the coding regions or introns in some genes. Evidence from deletion mutation studies has indicated that sequences downstream from the polyadenylation site are required for proper polyadenylation (44, 53–55). However, a consensus secondary regulatory element which could complement the hexanucleotide signal has not been identified yet (56). Whether the c-myc MAR at the 3'-end region and its specific recognition by the protein p25 may play a complementary role in regulating the polyadenylation which subsequently dictates the stability of the mature transcripts remains to be answered. Nevertheless, results presented here indicate that p25 is capable of simultaneously recognizing the poly(A) signal, the potential splicing site for polyadenylation and a topo II box-like sequence.

**ACKNOWLEDGMENTS**

We thank A. Geiser for artwork and Raymond Reeves for the gift of RP-HPLC purified murine HMG-I protein.

**REFERENCES**

Identification of a Nuclear Matrix-associated Region of the c-myc Protooncogene and Its Recognition by a Nuclear Protein in the Human Leukemia HL-60 Cell Line


Updated version  Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/50/11/3199

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.
Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.
Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.