Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 9682–9686, December 1989
Biochemistry

Ribonucleoprotein and protein factors bind to an H-DNA-forming c-*myc* DNA element: Possible regulators of the c-*myc* gene

(transcription element/DNA conformer/gene regulation)

TERRI L. DAVIS, ANTHONY B. FIRULLI, AND ALAN J. KINNIBURGH*

Department of Human Genetics, Roswell Park Memorial Institute, 666 Elm Street, Buffalo, NY 14263

Communicated by E. Racker, August 30, 1989 (received for review July 3, 1989)

ABSTRACT **We have located a positive, cis-acting DNA sequence element within the 5′ flanking DNA of the c-*myc* gene (−125 base pairs). This DNA sequence element has a large purine-pyrimidine strand asymmetry and can assume the H-DNA conformation. A factor with the properties of a ribonucleoprotein (RNP) interacts with this DNA region. The interaction of the c-*myc* DNA sequence element and the RNP involves an RNase H-sensitive mechanism and, therefore, may involve an RNA·DNA hybrid. In addition, a protein factor(s) binds to this DNA sequence element. DNA footprinting and mutant oligonucleotide binding/competition assays implicate a punctate, poly(G·C) recognition/binding sequence for the RNP factor, whereas the major protein factor requires two ACCCT sequence motifs for maximal binding. These results suggest that RNP and protein factors act as positive transcriptional regulators of the c-*myc* gene, perhaps by altering DNA topology.**

Several genes have been identified that regulate growth and differentiation. One such gene is the protooncogene c-*myc*. Enforced expression of c-*myc* in cultured mouse erythroleukemia cells disrupts differentiation and allows continued growth (1–3). When the c-*myc* gene is linked to the immunoglobulin μ enhancer, and made a transgene in mice, a pre-B cell hyperplasia results. A large percentage of these transgenics progress to a B-cell lymphoma (4, 5). c-*myc* antisense oligonucleotides inhibit cellular growth and induce differentiation in the human promyelocytic leukemia cell line HL-60 (6). These results demonstrate a direct role for c-*myc* in the regulation of cellular growth and an indirect role in cellular differentiation.

Sequence-specific DNA binding proteins regulate eukaryotic transcription (7–12). Although several protein factors have been shown to bind to the c-*myc* gene, the binding of these factors has not shown a correlation with the activity of the c-*myc* gene (13–15).

We, and others, have observed non-B DNA structures in the 5′ flanking region of the c-*myc* gene (ref. 16; T.L.D. and A.J.K., unpublished results). One of the single-strand nuclease-sensitive sites in the c-*myc* gene maps near and may correspond to a DNase I-hypersensitive site termed III_1 (17). DNase I sensitivity at the III_1 site disappears when cells become committed to terminal differentiation, a time when c-*myc* transcription is turned off (18). These data imply that the III_1 nuclease-hypersensitive site is a cis-acting regulatory element of the c-*myc* gene. Therefore, we examined the role of this DNA region in c-*myc* expression.

MATERIALS AND METHODS

c-*myc* Fusion Gene Constructs, Gene Transfection, and Chloramphenicol Acetyltransferase (CAT) Assays. Deletions were made in a subclone of the 866-base-pair (bp) *Pvu* II fragment containing exon I and 353 bp of 5′ flanking DNA. This subclone was digested with *Sma* I, and deletions were created with BAL-31 exonuclease and were sequenced by the dideoxy method (19). CAT constructs were transfected into HeLa cells as described (20). CAT assays were performed as described (21).

Oligonucleotide Preparation, Nuclear Extract Preparation, Gel Retardation Assay, and Phenanthroline/$CuSO_4$ Footprinting. Oligonucleotides were prepared using an Applied Biosystems oligonucleotide synthesizer. Nuclear extracts were prepared as described by Prywes and Roeder (22) except that phenylmethylsulfonyl fluoride and NaH_2PO_4 were added to each solution to a final concentration of 0.4 mM and 10 mM, respectively. Dialysis buffer was prepared without EDTA but with 1.5 mM $MgCl_2$. Nuclear extracts containing 1.0 μg of calf thymus DNA, 1.0 μg of *Alu* I- and *Hae* II-digested pUC13 DNA, and 1–5 ng of ^{32}P-labeled oligonucleotide were incubated for 30 min at 22°C. Band shift assays were then performed (23). 1,10-Phenanthroline/copper ion cleavages were performed *in situ* as described by Kuwabara and Sigman (24).

RESULTS

The S1 Nuclease-Sensitive DNA Region is a cis-Acting, Positive Transcription Element. Hay *et al.* (14) showed that a 192-bp deletion of sequences between −353 and −101 reduced c-*myc* gene expression, particularly from the P_1 promoter. We had mapped a single-strand nuclease-sensitive region to sequences around −125 bp (data not shown). To examine the role of the S1 nuclease-sensitive element in the expression of the c-*myc* gene, we prepared small deletions in this region and tested their promoter strength using the CAT gene as a reporter (21) (Fig. 1*A*). HeLa cells were transfected with these CAT gene fusions and CAT activity was assayed 72 hr later (Fig. 1*B*). The CAT activities were corrected using a cotransfected β-galactosidase internal control. The S1 nuclease sensitivity of each deletion was also assayed (Fig. 1*B*, lower panel). A deletion of 6 bp is 53% as active as the wild-type c-*myc* fusion gene and retains full S1 nuclease sensitivity (Fig. 1*B*). The 11-bp deletion has weak S1 nuclease cutting and retains 26% promoter activity. The 42- and 65-bp deletions have lost detectable S1 nuclease sensitivity and have 31% and 35% of wild-type CAT activity, respectively (Fig. 1*B*). This nuclease-sensitive DNA region behaves as a positive cis-acting transcription element.

Nuclear Factors Bind to the Nuclease-Sensitive, cis-Acting DNA. We assayed nuclear extracts for factors that bind to this DNA region. An oligonucleotide that is homologous to 25 bp of this sequence was synthesized (with *Bam*HI and *Bgl* II linkers) (Fig. 1*A*, see boxed region). Nuclear extracts were prepared from growing HL-60 cells, HL-60 cells induced to differentiate with dimethyl sulfoxide (DMSO) for 3 hr, and

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: NSE, nuclease-sensitive element; RNP, ribonucleoprotein; CAT, chloramphenicol acetyltransferase; DMSO, dimethyl sulfoxide.

*To whom reprint requests should be addressed.

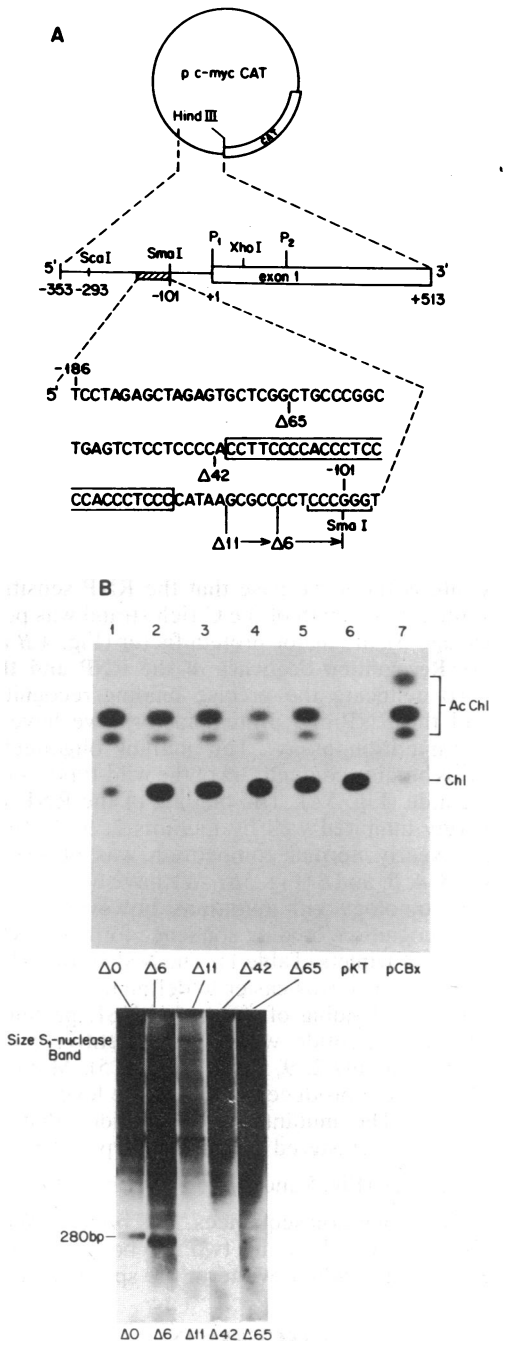

FIG. 1. c-*myc* deletions that remove portions of the S1 nuclease-sensitive region: Their activity as promoters in c-*myc*–CAT fusion genes. (*A*) Deletions were made as described in the text. The boxed area is the region homologous to a synthetic oligonucleotide used in Figs. 2, 3, and 5. (*B*) The CAT constructs were transfected into HeLa cells and CAT assays were performed. Chl, chloramphenicol; AcChl, acetylated chloramphenicol. Below the CAT assays is the S1 nuclease assay. The various c-*myc* deletions were digested with S1 nuclease and subsequently digested with *Hin*dIII to release the S1 nuclease/*Hin*dIII-cut DNA fragment (280 bp for the wild type). This DNA was end-labeled and electrophoresed as described (16).

HL-60 cells induced to differentiate for 3 days. In only the latter case is c-*myc* transcription turned off (18). The band shift assay was used to examine nuclear factor–oligonucleotide binding (23). Three shifted bands were observed in extracts from growing cells (Fig. 2, labeled 1, 1′, and 2). Each is reduced but still present in nuclear extracts from HL-60 cells treated for 3 hr with DMSO. After 3 days of DMSO treatment, a further reduction of bands 1 and 1′ is observed and band 2 is no longer detectable (Fig. 2). This reduction is not due to a general loss of nuclear factors since factors that bind to the c-*fos* serum-responsive element are at equivalent levels in all three nuclear extracts (data not shown). Qualitatively, factor binding is positively correlated with the transcriptional activity of the c-*myc* gene (18). Therefore, these factors are probably positive trans-acting regulators of c-*myc* gene expression. We henceforth call this c-*myc* transcription element the nuclease-sensitive element (NSE).

To test the nature of these factors, RNase A, RNase H, or proteinase K was added to nuclear extracts simultaneously with the NSE oligonucleotide. The results of band shift experiments demonstrate that at least two types of complexes form (Fig. 3). Band 2 is an RNase H-sensitive factor–NSE oligonucleotide complex (Fig. 3). The disruption of band 2 by RNase H suggests a base-paired RNA·DNA oligonucleotide. Since the oligonucleotide has been gel purified and 3′ end-labeled, labeled single-stranded oligonucleotides should not be present. However, the short NSE oligonucleotide may be denatured by other factors within the extracts.

Since proteinase K digestion disrupts all three NSE oligonucleotide–factor complexes the band 2 complex must also depend on proteins for its formation (Fig. 3*A*). These data imply that band 2 is a RNP–NSE oligonucleotide complex (Fig. 3*A*). Further evidence of a RNP–NSE oligonucleotide complex was revealed when extracts were treated with RNase A. Here band 2 disappears and a slower migrating band appears, band 2′ (Fig. 3*A*). Band 2′ is apparently an RNase A-generated artifact since we observe a similarly retarded band when a random oligonucleotide is assayed (see below).

Two additional experiments were performed to confirm that complex 2 formation is RNA dependent. (*i*) Disruption of complex 2 by RNase A was assayed using a specific RNase inhibitor (RNasin, Promega) (Fig. 3*B*). Disruption of complex 2 by RNase A is prevented when the RNase inhibitor is present. The smearing of probe (Fig. 3*B*, lanes 3 and 4), which obscures the band 1 and 1′ complexes, is an artifact generated by RNase A (see below). These results show that a contaminating enzymatic activity cannot account for the disruption of complex 2. (*ii*) Partially purified RNP factor bound to the c-*myc* NSE oligonucleotide. RNP binding remains RNase A

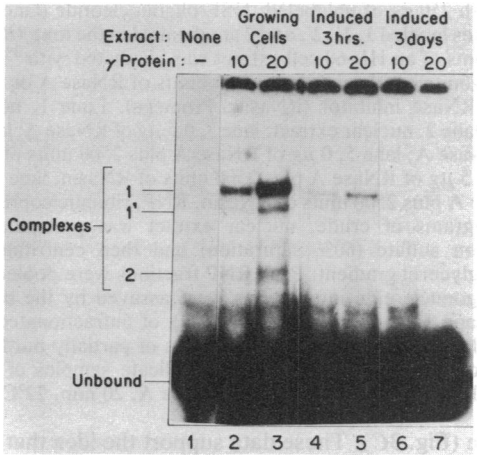

FIG. 2. An oligonucleotide homologous to the c-*myc* S1 nuclease-sensitive element forms complexes with nuclear factors from growing HL-60 cells. A band shift assay using nuclear extracts from HL-60 cells was performed. The assays were performed in the absence of extract (lane 1); in the presence of extracts from growing cells, 10 μg and 20 μg (lanes 2 and 3, respectively); in the presence of extracts from cells induced with DMSO for 3 hr, 10 μg and 20 μg (lanes 4 and 5, respectively); and in the presence of extracts from cells induced with DMSO for 3 days, 10 μg and 20 μg (lanes 6 and 7, respectively).

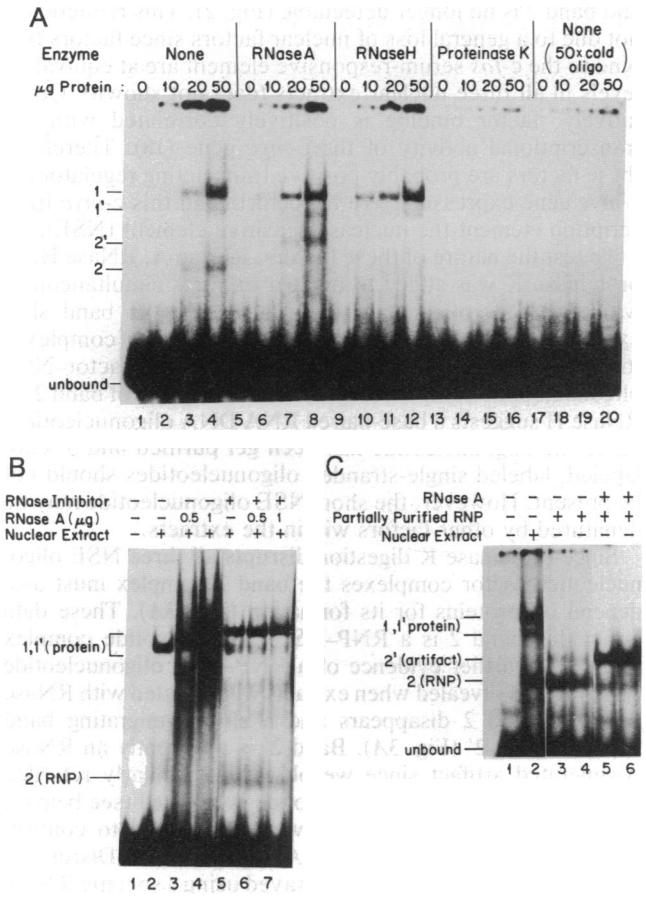

FIG. 3. Enzyme sensitivity of the NSE oligonucleotide–nuclear extract complexes. (*A*) Increasing amounts of a growing HL-60 cell nuclear extract (0, 10, 20, and 50 μg) were incubated with 5 ng of ^{32}P-labeled NSE oligonucleotide and scored for oligonucleotide–factor complexes by the band shift assay. Five sets of samples were analyzed. One set had no further additions (lanes 1–4), one set was treated with 4.0 μg of RNase A (lanes 5–8), one set was treated with 2.0 units of RNase H (lanes 9–12), one set was treated with 10 μg of proteinase K (lanes 13–16), and one set was incubated simultaneously with 250 ng of unlabeled, NSE oligonucleotide (lanes 17–20). Complexes labeled 1, 1', 2', and 2 are detailed in the text. (*B*) Twenty micrograms of an HL-60 cell extract was incubated with ^{32}P-labeled NSE oligonucleotide and various amounts of RNase A or RNase A plus an RNase inhibitor (RNasin, Promega). Lane 1, no nuclear extract; lane 2, nuclear extract; lane 3, 0.5 μg of RNase A; lane 4, 1.0 μg of RNase A; lane 5, 0 μg of RNase A plus 2500 units of RNasin; lane 6, 0.5 μg of RNase A plus 2500 units of RNasin; lane 7, 1.0 μg of RNase A plus 2500 units of RNasin. RNP, ribonucleoprotein. (*C*) Six milligrams of crude, nuclear extract was precipitated with ammonium sulfate (60% saturation) and then centrifuged on a 10–30% glycerol gradient. Peak RNP fractions were pooled. Factor NSE–oligonucleotide interactions were assayed by the band shift assay. Lane 1, no extract; lane 2, 50 μg of unfractionated nuclear extract; lanes 3 and 4, duplicate samples of partially purified RNP (≈5 μg of protein); lanes 5 and 6, duplicate samples of partially purified RNP treated with 4 μg of RNase A, 20 min, 22°C.

sensitive (Fig. 3*C*). These data support the idea that RNA is an integral component of the band 2 complex.

Band 1 and band 1' are protein–NSE oligonucleotide complexes since proteinase K disrupts these complexes but not RNase A or RNase H (Fig. 3*A*). None of the enzymes bind to the NSE oligonucleotide (Fig. 3*A*, lanes 5, 9, and 13). To ensure that the factor NSE–oligonucleotide complexes are dependent on the NSE sequence and not its high GC content (66%), a 35-bp random sequence oligonucleotide of 66% GC content was tested for factor binding. We observed no oligonucleotide complexes (data not shown). No random oligonucleotide–factor complex is formed upon RNase H treatment. However, RNase A treatment generates an oligonucleotide–factor complex (data not shown). Perhaps other cellular RNPs are present in our nuclear extracts and, when digested with RNase A, their protein moieties may interact with other polyanions such as oligonucleotides.

DNA Protection by the RNP and NSE Protein. To examine the NSE interaction with the RNP and protein factors, we performed phenanthroline/Cu^{2+} protection experiments *in situ* after band shift assays (24). The band 1' (minor NSE-binding protein) was not examined. Also a RNP "dimer" was isolated and analyzed (data not shown). The RNP weakly protected five nucleotides of the G-rich strand from cleavage (Fig. 4 *A* and *C*). The RNP protected the C-rich strand from cleavage over 28 nucleotides of NSE, and the RNP dimer showed a greater protection of this region (Fig. 4 *B* and *C*). The RNP produced a hypersensitive cleavage site at both bases of a G·C base pair (Fig. 5 *B* and *C*). In contrast to the RNP factor, the major NSE-binding protein did not protect the G-rich strand (Fig. 4 *A* and *C*). When the C-rich strand was protected, a hypersensitive cleavage site was found. This cleavage site is the same base that the RNP sensitized. In addition, the entire length of the C-rich strand was protected from cleavage by the major protein factor (Fig. 4 *B* and *C*).

Binding/Recognition Sequence of the RNP and the NSE Protein. To delineate the precise binding/recognition sequences of the RNP and protein factors, we have used a binding/competition assay. Ten mutant oligonucleotides were used as binding competitors of the wild-type c-*myc* NSE oligonucleotide (Fig. 5*A*). The binding of the RNP was not competitively inhibited well by mutants 2, 9, 3, 3A, and 7 (Fig. 5*B*). Nearly normal competition was observed with mutants 1, 8, 4, 5, and 6 (Fig. 5*B*). We have not saturated the 25-bp NSE homology with mutations; however, we propose a minimal recognition/binding sequence for this RNP based on GC base-pair triplets (Table 1). The NSE-protein binding/recognition sequence was easier to define using our panel of 10 mutants. The binding of the major NSE protein to the wild-type oligonucleotide was *not* competitively inhibited efficiently by mutants 2, 9, 3, and 5 (Fig. 5). Mutant oligonucleotides 8 and 6 produced intermediate levels of competition (Fig. 5). The mutant oligonucleotides that did not compete well are clustered within two repeating sequence motifs ($\frac{\text{ACCCT}}{\text{TGGGA}}$) (Fig. 5 and Table 1). We have termed these NSE protein recognition sequences "AC boxes." Within the NSE oligonucleotide there are two AC boxes separated by four CG base pairs, which we term the spacer (Fig. 5).

DISCUSSION

A cis-Acting Transcription Element of the c-*myc* Gene Binds Nuclear Factors. Others have noted an unusual c-*myc* DNA element with single-strand nuclease hypersensitivity (16). These authors hypothesized that an RNA·DNA hybrid or a single-strand RNA·double-stranded DNA triplex may form and participate in the regulation of the c-*myc* gene (25). We have shown that this nuclease-sensitive c-*myc* DNA region acts as a cis-acting positive transcription element *in vivo* and binds a factor with the properties of a RNP (Fig. 1). The binding of the RNP factor may involve base-pairing since RNP binding is sensitive to RNase H. A protein factor(s) also binds to the NSE oligonucleotide (Figs. 2 and 3). The protein and the RNP binding activities are at highest levels in growing cells and decrease 3 hr after induction of differentiation in HL-60 cells (Fig. 2). Small amounts of protein–NSE oligonucleotide complexes and no RNP–NSE oligonucleotide complexes are observed 3 days after induction of differentiation (when c-*myc* transcription has been turned off) (18) (Fig. 2). Therefore the RNP and protein factors are likely to

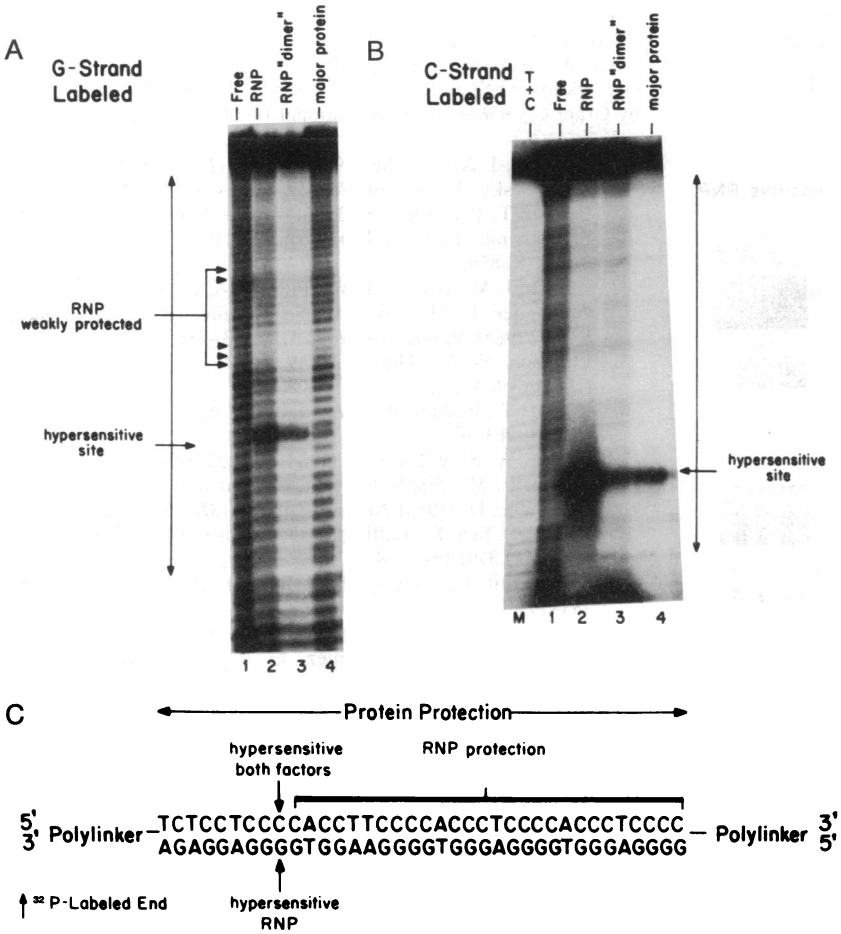

FIG. 4. Phenanthroline/$CuSO_4$ DNA footprinting of the NSE-binding protein and RNP factors. *In situ* phenanthroline/$CuSO_4$ cleavage of the NSE-binding protein and RNP factors was performed. Cleaved samples, along with sequencing markers, were electrophoresed on a 5% polyacrylamide/7 M urea gel (30 × 40 cm). (*A*) Seventy-two-base-pair *Eco*RI/*Xho* I restriction fragment protection with G-rich strand labeled. Lane 1, free (unbound) DNA; lane 2, RNP-bound DNA; lane 3, RNP dimer-bound DNA; lane 4, major NSE-binding protein–DNA complex. (*B*) Seventy-two-base-pair *Eco*RI/*Xho* I restriction fragment protection with C-rich strand labeled. Lane M, T+C sequence markers; lane 1, free (unbound) DNA; lane 2, RNP-bound DNA; lane 3, RNP dimer-bound DNA; lane 4, major NSE-binding protein–DNA complex. Each lane was loaded with the same amount of [^{32}P]DNA. (*C*) Summary of NSE factor protection/sensitivity. ↑, Sites of $CuSO_4$/phenanthroline hypersensitivity; ▲, sites weakly protected by the RNP factor; ←→, c-*myc* homologous region of the *Eco*RI/*Xho* I restriction fragment.

be positive trans-acting regulators of c-*myc* transcription. These factors are not the sole determinants of c-*myc* gene expression since negative cis-acting elements have been found 5′ to the NSE described here (13, 14, 26).

The DNA Binding/Recognition Sequences of the NSE Proteins and the NSE RNP. Binding/competition experiments defined the major protein binding/recognition sequence as ACCCTNNNNACCCT / TGGGANNNNTGGGA. We term this repeating sequence motif an "AC box" (Fig. 5 and Table 1). Mutations in either AC box can disrupt protein binding (Fig. 5 and Table 1). Therefore, two AC boxes appear necessary for maximal binding. The RNP has a more disperse recognition sequence involving GC base pairs (Fig. 5 and Table 1).

Protection from phenanthroline/Cu^{2+} cleavage was also used to assess the interaction of nucleotide sequences with the RNP and protein factors. The RNP and protein binding/recognition sequences are a subset of the DNA regions protected from phenanthroline/Cu^{2+} cleavage (Figs. 4 and 5, Table 1). The agreement of these two techniques lends support to the designation of the RNP as a DNA-binding factor.

DNA Topology and Transcriptional Regulation. The c-*myc* NSE can assume the H-DNA conformation *in vitro* (27).

Table 1. Proposed binding/recognition sequence of NSE-binding factors

NSE sequence	C	C	T	T	C	C	C	C	A	C	C	C	T	C	C	C	C	A	C	C	C	T	C	C	C
	G	G	A	A	G	G	G	G	T	G	G	G	A	G	G	G	G	T	G	G	G	A	G	G	G
Sequence requirement for RNP	?	?	–	?	?	?	?	?	–	+	+	?	?	+	?	?	–	?	?	–	?	–	?	?	+
												+													
Recognition/binding sequence of RNP									N	C	C	C	N	C	C	C	C	N	N	N	N	N	C	C	C
									N	G	G	G	N	G	G	G	N	N	N	N	N	N	G	G	G
												?			?	?		?	?		?		?	?	
Sequence requirement for major protein	?	?	–	?	?	?	?	?	±	+	+	?	+	±	?	?	–	?	?	+	?	±	?	?	–
Recognition/binding sequence of major protein									A	C	C	C	T	N	N	N	N	A	C	C	C	T			
									T	G	G	G	A	N	N	N	N	T	G	G	G	A			
									AC box					Spacer				AC box							

Proposed binding/recognition sequences for RNP and protein binding to NSE oligonucleotide. Data from Fig. 5 were used to determine whether the base-pair mutation within a mutant oligonucleotide is required for factor binding. If the mutant oligonucleotide competed as well as the wild-type oligonucleotide or if a 5-fold higher level of the mutant oligonucleotide was needed for a similar level of competition, we considered the base-pair mutation not needed for recognition/binding. These base pairs are designated –. If a >5-fold excess of mutant oligonucleotide was necessary for a level of competition that equals the wild-type oligonucleotide, then we considered the base-pair mutation to be needed for recognition/binding. The base pairs that are needed for binding are designated +. Mutant oligonucleotides that are intermediate in their effects on competition are designated ±.

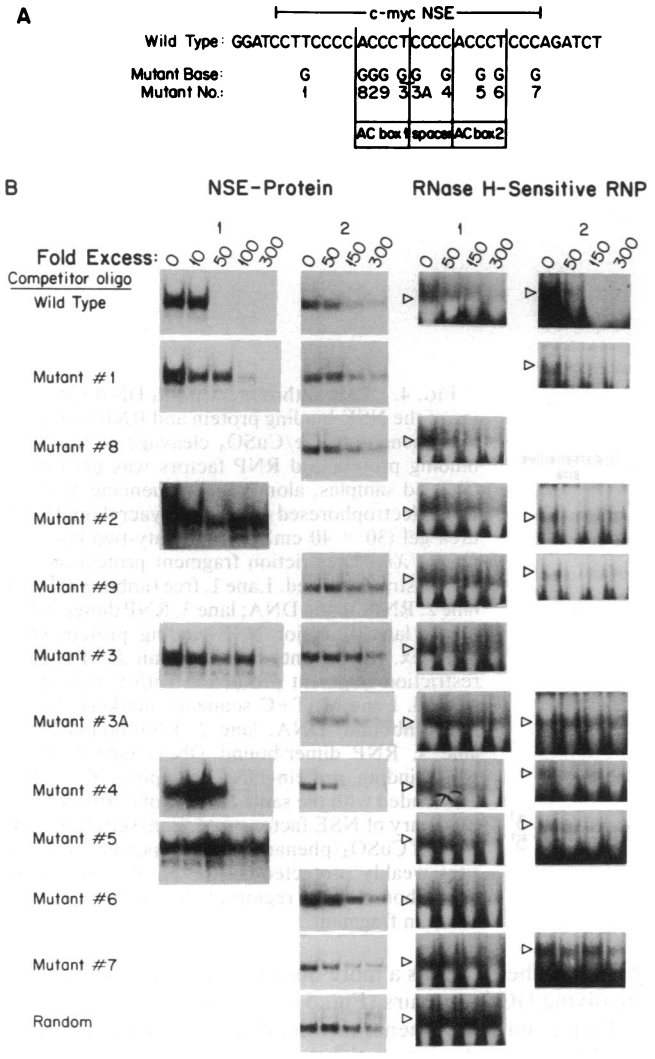

FIG. 5. Definition of the RNP and major protein binding/recognition sequence within the NSE oligonucleotide. (*A*) The c-*myc* NSE oligonucleotide, the region of homology, and mutant oligonucleotides are shown. The AC boxes and spacer are defined and discussed in the text. (*B*) Competition experiments utilizing the band shift assay. Eacn mutant oligonucleotide was incubated with nuclear extract (20 μg or 25 μg for experiments 1 and 2, respectively) for 30 min, ^{32}P 3′ end-labeled NSE oligonucleotide was added, and incubation was continued for 30 min. Numbers 1 and 2 refer to experiments performed with different extracts and labeled oligonucleotide preparations.

H-DNA is a triplex/single-stranded DNA structure (28–32). However, conditions within the cell may not favor an H-DNA conformation (28). Therefore, the binding of the RNP and protein factors to the c-*myc* NSE DNA region may induce or stabilize the H-DNA conformer *in vivo*. We hypothesize that the open H-DNA conformer would stimulate c-*myc* transcription since the levels of these factors are reduced when c-*myc* gene activity is reduced (Fig. 2). Given the widespread occurrence of nuclease-hypersensitive sites adjacent to RNA polymerase II-transcribed genes (33, 34), the interaction of protein and RNP factors with H-DNA-forming DNA regions may be a general feature of transcriptional regulation.

We acknowledge Drs. K. Gross, T. Torrey, M. Linskens, D. Kowalski, C. Wenner, and R. Umek for helpful comments on this work. We thank Nancy Frame for secretarial assistance and Donna Oleszek for excellent technical assistance. This work was supported by Grant CA 43661 from the National Institutes of Health.

1. Coppola, J. & Cole, M. (1986) *Nature (London)* **320,** 760–763.
2. Dmitrovsky, E., Kuehl, W. M., Hollis, G. F., Kirsch, I. R., Bender, T. P. & Segal, S. (1986) *Nature (London)* **322,** 748–750.
3. Prochownik, E. V. & Kukowska, J. (1986) *Nature (London)* **322,** 848–850.
4. Adams, J. M., Harris, A. W., Pinkert, A. A., Corcoran, C. A., Alexander, L. M., Cory, W. S., Palmiter, R. D. & Brinster, R. L. (1985) *Nature (London)* **318,** 533–538.
5. Langdon, W. Y., Harris, A. W., Cory, S. & Adams, J. M. (1986) *Cell* **47,** 11–18.
6. Holt, J. T., Redner, R. L. & Nienhuis, A. W. (1988) *Mol. Cell. Biol.* **8,** 963–973.
7. Dynan, W. S. & Tjian, R. (1983) *Cell* **32,** 669–680.
8. Staudt, L. M., Singh, H., Sen, R., Wirth, T., Sharp, P. A. & Baltimore, D. (1986) *Nature (London)* **323,** 640–643.
9. Singh, H., Sen, R., Baltimore, D. & Sharp, P. A. (1986) *Nature (London)* **319,** 154–158.
10. Scheidereit, C., Heguy, A. & Roeder, R. G. (1987) *Cell* **51,** 783–793.
11. Bauerle, P. A. & Baltimore, D. (1988) *Science* **242,** 540–546.
12. Yamamoto, K. K., Gonzalez, G. A., Biggs, W. H., III & Montiminy, M. R. (1988) *Nature (London)* **334,** 494–498.
13. Chung, J., Sinn, E., Reed, R. R. & Leder, P. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 7918–7922.
14. Hay, N., Bishop, J. M. & Levens, D. (1987) *Genes Dev.* **1,** 659–671.
15. Asselin, C., Nepveu, A., Wang, J.-Q. & Marcu, K. B. (1988) in *Growth Factors and Receptors*, ICN-UCLA Symposia, eds. Ross, R., Burgess, A. W. & Hunter, T. (Liss, New York), Vol. 102, pp. 239–251.
16. Boles, T. C. & Hogan, M. E. (1987) *Biochemistry* **26,** 367–376.
17. Siebenlist, V., Henninghausen, K., Battey, J. & Leder, P. (1984) *Cell* **37,** 381–391.
18. Siebenlist, U., Bressler, P. & Kelly, K. (1988) *Mol. Cell. Biol.* **8,** 867–874.
19. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
20. Maquat, L. E. & Kinniburgh, A. J. (1985) *Nucleic Acids Res.* **13,** 2855–2866.
21. Gorman, C. M., Moffat, L. F. & Howard, B. H. (1982) *Mol. Cell. Biol.* **2,** 1044–1051.
22. Prywes, R. & Roeder, R. G. (1987) *Mol. Cell. Biol.* **7,** 3482–3489.
23. Fried, M. & Crothers, D. M. (1981) *Nucleic Acids Res.* **9,** 6505–6525.
24. Kuwabara, H. D. & Sigman, D. S. (1987) *Biochemistry* **26,** 7234–7238.
25. Cooney, M., Czernuszewicz, G., Postel, E. H., Flint, S. J. & Hogan, M. E. (1988) *Science* **241,** 456–459.
26. Remmers, E. F., Yang, J.-Q. & Marcu, K. B. (1986) *EMBO J.* **5,** 889–904.
27. Kinniburgh, A. J. (1989) *Nucleic Acids Res.* **17,** 7771–7778.
28. Lyamichev, V. I., Mirkin, S. M. & Frank-Kamenetskii, M. D. (1986) *J. Biomol. Struct. Dyn.* **3,** 667–669.
29. Voloshin, O. N., Mirkin, S. M., Lyamichev, V. I., Belotserkovskii, B. P. & Frank-Kamenetskii, M. D. (1988) *Nature (London)* **333,** 475–476.
30. Hanvey, J. C., Shimizu, M. & Wells, R. D. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 6292–6296.
31. Htun, H. & Dahlberg, J. E. (1988) *Science* **241,** 1791–1795.
32. Htun, H. & Dahlberg, J. E. (1989) *Science* **243,** 1571–1576.
33. Larsen, A. & Weintraub, H. (1982) *Cell* **29,** 609–622.
34. Schon, E., Evans, T., Welsh, J. & Efstradiatis, A. (1983) *Cell* **35,** 837–848.