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Posttranscriptional gene regulation and specific binding of the nonhistone protein HMG-I by the 3′ untranslated region of bovine interleukin 2 cDNA

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ABSTRACT **The 3′ untranslated tail region (3′-UTR) of the cDNA of bovine interleukin 2 (bIL-2) acts as a lymphoid cell-specific gene regulatory element *in vivo* when ligated to the 3′ end of the "marker" bacterial gene coding for chloramphenicol acetyltransferase (CAT) and the hybrid fusion gene is introduced into bovine lymphoid cells by transfection. Evidence is also presented that the 3′-UTR with its conserved $(TATT)_n$ motif probably has multiple functions in lymphoid cells operating both at the chromosomal level, where the sequence may be involved in the specific binding of the nonhistone chromatin high mobility group protein HMG-I, and at the RNA level, where the conserved sequence is involved in selective posttranscriptional mRNA degradation by a lymphocyte-specific nuclease(s). These results suggest a complex *in vivo* role for the 3′-UTR of bIL-2 cDNA and the conserved $(TATT)_n$ sequences found within it. They also offer a plausible explanation for the high degree of conservation of similar A+T-rich sequences in the 3′-UTRs of many of the other immune-response and growth-regulatory genes of mammals.**

A common control feature shared by many lymphokine, cytokine, growth-factor, and immune-response genes of mammals is the dual operation of both transcriptional and posttranscriptional mechanisms to regulate their expression *in vivo* (1–8). From detailed analyses of the cDNAs and genomic molecular clones coding for some of these genes, certain of the DNA sequences involved in the cell-specific inducible promotion (9–11) and enhancement (12) of transcription, as well as sequences involved in posttranscriptional processes (2, 5, 13–15), have been identified. In this regard we (13), and others (14, 15), have recently identified long stretches (>20 nucleotides) of a highly conserved A+U-rich sequence commonly found in the 3′ untranslated tail region (3′-UTR), between the termination codon and the polyadenylylation signal, of the mRNAs of many of the known lymphokine, cytokine, and protoonco- genes. At the DNA level this conserved sequence appears to be composed of tandem repeats of an ancestral tetranucleotide (TATT) unit that has undergone limited divergence during the evolution of these genes (13). Significantly, not all of the genes known to be induced during the immune activation of lymphocytes [e.g., the interleukin-2 (IL-2) receptor (16), the transferrin receptor (17), and certain proteases (18)] have such canonical sequence motifs in their 3′-UTRs, suggesting that these highly conserved $(TATT)_n$ sequences may serve specific regulatory or other roles in those genes that do possess them. Here we report on multiple functions found to be associated with the 3′-UTR of the cDNA of bovine interleukin-2 (bIL-2).

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MATERIALS AND METHODS

Cell Culture and DNA Transfections. Adherent strains of IL-2-dependent bovine lymphoid cells (e.g., E3 and 27X) were derived and maintained as previously described (13), as was the monkey fibroblastic cell line CV-1. Plasmid DNAs were introduced into cells either by an optimized calcium phosphate transfection procedure or by a DEAE-dextran procedure (19) in the presence of 80 μM chloroquine (20). After transfection, cells were washed with fresh medium (modified Dulbecco's medium containing 10% fetal calf serum) and cultured in either normal medium or medium containing phytohemagglutinin (PHA; 8 μg/ml; PHA-L from Sigma) for 15 hr at 37°C. At the end of this incubation the experimental cells were washed free of PHA and, in some cases, exposed for 4 hr to cycloheximide (CHX) (50 μg/ml; Sigma). After this short treatment, the cells were washed free of CHX and incubated in normal medium for an additional 26 hr before harvesting for assay. Assays for chloramphenicol acetyltransferase (CAT) activity in transfected cells followed published procedures (21).

Plasmid Construction and "Riboprobe" RNAs. Plasmids pSV2cat.3′bIL-2 and pSV2cat.$(TATT)_{13}$ were constructed as described in the text, starting with the parent plasmids pbIL-2 (13) and pSV2cat (21). Synthetic double-stranded DNA polymers [e.g., $(TATT)_{13}$ and others] were synthesized as complementary single-stranded oligonucleotides with an Applied Biosystems 380A DNA synthesizer. Upon annealing, the synthetic double-stranded DNA constructs were ligated into the multiple cloning sites of Bluescribe M13+ and M13− plasmid vectors (Stratagene) and the nucleotide sequence of the synthetic inserts was confirmed by DNA sequencing employing the chain-termination method (22). For use, either the inserts in the Bluescribe vectors were excised with appropriate restriction enzymes or high specific activity [α-^{32}P]dCTP-labeled riboprobe RNA transcripts (10^8–10^9 cpm/μg) of the inserts were produced by using either T3 or T7 RNA polymerase.

High Mobility Group I (HMG-I) Protein Isolation, DNA-Binding Assays, and DNase I "Footprinting." HMG-I protein was isolated from either Friend erythroleukemia or mouse ascites cells and purified by ion-pair reverse-phase HPLC (23). DNase I footprints were prepared by the method of Pruijn *et al.* (24).

Nuclease Assays. Nuclease activities in nuclear extracts from bovine lymphoid and other cells were assayed by homogenization of freshly isolated nuclei (25) in a buffered solution (10 mM Tris·HCl, pH 7.2/20 mM KCl/2 mM $MgCl_2$/2 mM $CaCl_2$/1 mM ATP) followed by addition of ^{32}P-labeled riboprobe RNA transcripts to the extracts and the

Abbreviations: IL-2, interleukin 2; bIL-2, bovine IL-2; PHA, phytohemagglutinin; HMG, high mobility group (nonhistone chromatin proteins); 3′-UTR, 3′ untranslated tail region; CHX, cycloheximide; CAT, chloramphenicol acetyltransferase.

mixture was incubated at 37°C. At intervals, aliquots were taken and the amount of acid-insoluble (10% trichloroacetic acid, 5°C) radioactive material remaining in the extracts was determined. Nuclear extracts of CHX-treated cells were made immediately after the 4-hr treatment of cells with the protein synthesis inhibitor.

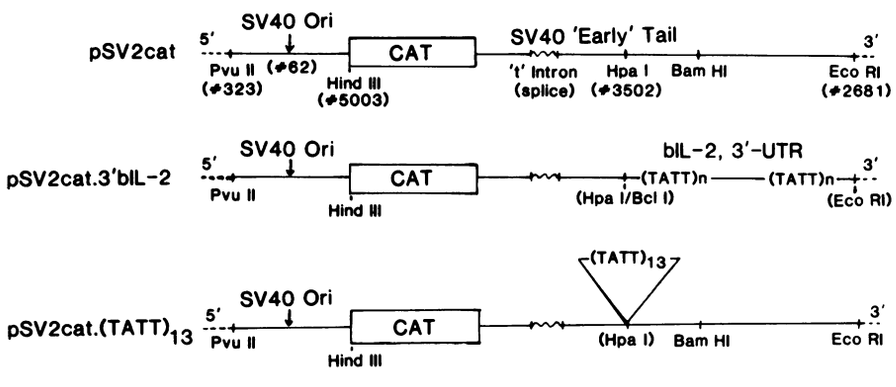
RESULTS

Effect of the 3'-UTR and $(TATT)_n$ Sequences on Expression of CAT-Fusion Genes. To study the function of the 3'-UTR of bIL-2 and its conserved $(TATT)_n$ sequences, the following plasmids were constructed (Fig. 1*A*). In one construct, pSV2cat.3'bIL-2, the entire 3'-UTR (295 bp) of bIL-2 (13) was inserted into the plasmid vector pSV2cat (21) between the *Hpa* I (nucleotide residue 3502) and one of the two *Eco*RI sites (residue 2618). In a second construct, pSV2cat.$(TATT)_{13}$, a synthetic 5'-$(TATT)_{13}$-3' oligonucleotide (A+T-rich consensus sequence; ref. 13), was inserted into the *Hpa* I site (residue 3502) in the pSV2cat vector. The different hybrid pSV2cat fusion plasmids were individually introduced into various adherent bovine lymphoid cell strains (e.g., E3 and 27X), as well as into CV-1 fibroblasts, by standard DNA transfection techniques and, after 45 hr, extracts of the transfected cells were assayed for CAT activity (Fig. 1*B*).

Preliminary experiments indicated that the 3'-UTR of bIL-2 lacked "enhancer-like" (26) activity in transfection assays (data not shown). In subsequent transfection experiments we found that while the CAT-fusion gene plasmids pSV2cat.3'bIL-2 and pSV2cat.$(TATT)_{13}$ were both active when individually introduced into control CV-1 monkey fibroblast cells, no CAT activity was detected when either of these plasmids was transfected into the E3 strain of bovine lymphoid cells (Fig. 1*B* and unpublished data). This was true whether or not the recipient cells had been stimulated with the mitogen PHA (Fig. 1*B*). On the other hand, control experiments indicated that the normal parent plasmid pSV2cat transfected into the E3 bovine lymphoid cells (with or without PHA treatment) did express detectable CAT activity (Fig. 1*B*). These results indicated that both the 3'-UTR and the $(TATT)_{13}$ sequences inserted 3' to the bacterial CAT gene completely and selectively repressed CAT activity in transfected bovine lymphoid cells but not in transfected nonlymphoid CV-1 fibroblasts.

Cycloheximide Induction of CAT Activity in Lymphoid Cells. To further investigate the inability of recipient bovine lymphoid cells to express CAT-fusion genes containing inserted $(TATT)_n$ repeat sequences in their 3'-UTRs, we attempted to overcome this blockage by treatment of the transfected lymphoid cells with CHX, a compound that

A



B

PLASMID	CELL TYPE	TREATMENT	ENZYME ASSAY	% CONVERSION
pSV2cat	CV-1	NONE		99
pSV2cat.3'bIL-2	CV-1	NONE		25
pSV2cat.$(TATT)_{13}$	CV-1	NONE		22
Sperm DNA	E3	NONE		0
pSV2cat	E3	NONE		41
pSV2cat.3'bIL-2	E3	NONE		0
pSV2cat.$(TATT)_{13}$	E3	NONE		0
pSV2cat	E3	+ PHA		55
pSV2cat.3'bIL-2	E3	+ PHA		3
pSV2cat.$(TATT)_{13}$	E3	+ PHA		0
pSV2cat.3'bIL-2	E3	+ PHA & CHX		26

FIG. 1. Transient expression in transfected cells of hybrid CAT-fusion gene constructs. (*A*) Diagram of the CAT gene plasmid expression vector constructs used in transfections of bovine E3 lymphoid cells and monkey CV-1 fibroblast cells. SV40 Ori, origin of replication of simian virus 40 DNA. (*B*) Assays of CAT activity in recipient transfected cells given different regimes of treatment. The type of expression vector (Plasmid) transfected into a given host cell strain (Cell type) is indicated. Also shown are assays for CAT activity in cell extracts (Enzyme assay) and the efficiency of conversion of unacetylated to acetylated forms of [^{14}C]chloramphenicol by the extracts (% conversion).

inhibits translation by interfering with peptide bond formation. Appropriate treatment of activated human lymphocytes with CHX is known to result in "superinduction" of both IL-2 mRNA and secreted IL-2 protein (7, 8). CHX also induces the production of human β-interferon in normally nonproducing mouse NIH 3T3 cells transfected with the human β-interferon gene (5). Bovine lymphoid cells transfected with pSV2cat.3'bIL-2 (Fig. 1*B*) responded to a short (4-hr) exposure to CHX by the production of CAT enzyme activity to a level comparable to that found in untreated CV-1 fibroblast cells transfected with the same plasmid (Fig. 1*B*). Thus, the recipient E3 bovine lymphoid cells treated with CHX clearly showed an *in vivo* biological induction of enzyme activity of CAT-fusion genes containing the 3'-UTR of bIL-2. The amount of CAT enzyme induction by CHX varied with different recipient bovine lymphoid cell strains and with different regimes of PHA and CHX treatment. Regardless of the actual amount of stimulation, however, all of these results clearly demonstrate that CAT-fusion genes containing the bovine IL-2 3'-UTR sequence can be expressed in transfected bovine lymphoid cells. Nevertheless, they also reveal that the 3'-UTR sequences have a strong suppressive effect on fusion-gene expression *in vivo* that is reversed or bypassed by appropriate CHX treatment of transfected cells.

Selective Nuclease Degradation of $(UAUU)_n$-Containing mRNAs. Induction of gene expression by inhibitors of protein synthesis, such as CHX, can occur by changes at both the transcriptional and posttranscriptional levels. In the case of superinduction of IL-2 by CHX treatment in human lymphocytes, it has been postulated that a short-lived protein "repressor" molecule whose synthesis is prevented by exposure to the drug might be involved (7). This repressor most likely operates at a posttranscriptional level (7, 8). Effects of CHX on posttranscriptional processes during the induction of human β-interferon (2–4), human γ-interferon (5), and human granulocyte/macrophage colony-stimulating factor (15) have also recently been reported.

As demonstrated in Fig. 1*B*, a posttranscriptional mode of action of CHX on transfected bovine lymphocytes also seemed likely since mRNA transcription from the CAT gene in both the pSV2cat.3'bIL-2 and pSV2cat.$(TATT)_{13}$ plasmids was under the control of the constitutively active simian virus 40 early promoter sequences (21, 27). Additionally, the required location of the A+T-rich sequences in the 3'-UTR of the CAT-fusion genes suggested that the major effects of these sequences on gene expression were exerted posttranscriptionally. Further support for this suggestion came from the fact that the parent pSV2cat vector itself was active in lymphocytes (Fig. 1*A*) and from the observation of an orientation-dependent effect on CAT gene expression of the synthetic $(TATT)_{13}$ oligonucleotide inserted 3' to the bacterial gene in the *Hpa* I site of parent vector pSV2cat (data not shown).

We therefore investigated the possibility that bovine lymphoid cells might contain a labile nuclease (whose synthesis could be inhibited by CHX treatment) that could selectively degrade mRNAs containing repetitive $(UAUU)_n$ elements in their 3'-UTR. The results shown in Fig. 2 demonstrate that such an enzymatic activity exists in nuclear extracts prepared from activated bovine lymphoid cells growing in culture. In these experiments, Bluescribe (Stratagene) ^{32}P-labeled RNA riboprobe transcripts of various gene constructs were isolated and incubated with nuclear extracts from either bovine lymphoid or CV-1 fibroblast cells. All of the riboprobe transcripts containing the repetitive $(UAUU)_n$ sequences (including the isolated RNA copies of the 3'-UTR sequence alone) were selectively degraded by nuclear extracts from the lymphoid cells, and this preferential degradation was substantially reduced by CHX treatment of

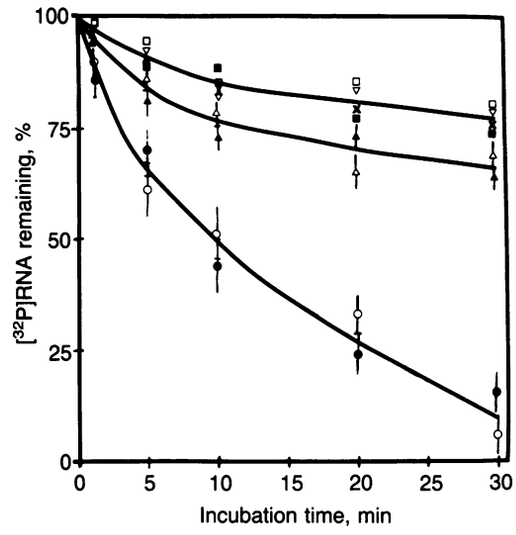


FIG. 2. Stability of ^{32}P-labeled riboprobe RNA transcripts in nuclear extracts. The graph plots the percentage of acid-insoluble (5% trichloroacetic acid, 0°C) radioactive RNA remaining as a function of incubation time in nuclear extracts. ●, Complete bIL-2 RNA transcript incubated in bovine E3 lymphoid cell extract; ○, transcript of the 295-bp fragment corresponding to the 3'-UTR of the bIL-2 gene incubated in E3 extract; □, transcript of the 496-bp fragment corrresponding to the 5' coding region of bIL-2 incubated in E3 extract; ▲, transcript of complete bIL-2 gene incubated in extract of CHX-treated E3 cells; △, transcript of the 3'-UTR fragment incubated in extract of CHX-treated E3 cells; ■, transcript of complete bIL-2 gene incubated in monkey CV-1 fibroblast cell extract; x, transcript of the 5' coding fragment incubated in CV-1 extract; ▽, transcript of the 3'-UTR fragment incubated in CV-1 extract. Bars indicate ±SEM for four independent experiments.

the lymphoid cells prior to preparation of the extracts (Fig. 2). Both heat and protease treatment of the lymphoid nuclear extracts eliminated this selective RNA degradation (data not shown), consistent with the notion that a protein nuclease was involved. The absence of such a highly selective degradative activity in the nuclear extracts of monkey CV-1 fibroblast cells may reflect the existence of lymphoid cell-specific regulatory mechanisms for controlling lymphokine gene expression posttranscriptionally, as has been postulated by others (28). These results do not, however, argue against the possible existence in the cytoplasm of these cells of additional mRNA-specific factors that could also be involved in gene regulation.

Specific Binding of Nonhistone Chromatin Protein HMG-I to the 3'-UTR. Sequence-specific nonhistone chromatin proteins that bind to DNA regulatory regions often play crucial roles in the control of gene expression. To investigate the possibility that such sequence-specific proteins might be involved in binding to the 3'-UTR regulatory region of bIL-2 we incubated full-length cDNA (791 bp) in nonhistone chromatin protein extracts isolated from murine ascites cell nuclei by 0.35 M NaCl elution (23). We found that this crude nuclear extract did indeed contain a protein factor(s) that specifically bound with high affinity to the 3'-UTR (but not the 5' coding region) of the bIL-2 cDNA *in vitro* (data not shown). Subsequent fractionation of the crude extract revealed that the specific 3'-UTR-binding factor found in this preparation was a member of the high mobility group (HMG) nonhistone nuclear proteins (ref. 25; unpublished results). We have identified this specific binding factor and report here that it is the nonhistone chromatin protein HMG-I (ref. 23; see below).

Murine HMG-I purified to homogeneity by reverse-phase HPLC (23) was bound *in vitro* to either ^{32}P-end-labeled full-length copies of bIL-2 cDNA or appropriately cleaved 5' protein-encoding and 3'-UTR noncoding subfragments of this

cDNA, in the presence of a 4000-fold excess of unlabeled competitor poly(dI·dC) DNA. The resulting specific protein·DNA complexes were resolved by polyacrylamide gel electrophoresis at low ionic strength and visualized by autoradiography. The results of such DNA fragment electrophoretic mobility shift assays indicated that purified HMG-I specifically bound to multiple sites in the noncoding 3′-UTR segment of bIL-2 cDNA but did not specifically bind in the 5′-coding region (data not shown).

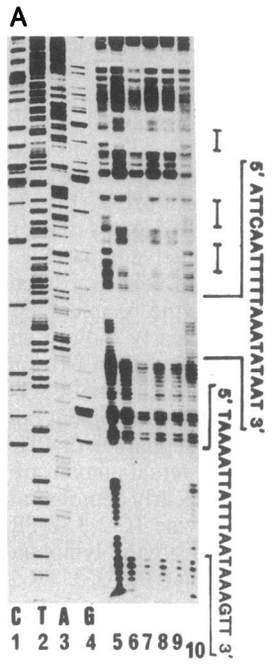
HMG-I-Binding Sites in the 3′-UTR Detectable by DNase I Footprinting. Using limited DNase I digestions, we identified specific HMG-I-binding sites along the entire bIL-2 cDNA sequence. Fig. 3*A* shows the pattern of nuclease sensitivity obtained when a restriction fragment containing only the 3′-UTR was digested with DNase I in the presence of increasing amounts of added HPLC-purified HMG-I and a vast (4000-fold) excess of nonspecific *Escherichia coli* competitor DNA. The added pure protein protects a number of discrete regions of the 3′-UTR from nuclease digestion. These regions are shown in Fig. 3. They are all A+T-rich stretches of DNA and include the highly conserved $(TATT)_n$ sequences. In contrast to the 3′-UTR fragment, the 496-bp DNA fragment containing the 5′ protein-encoding region of the bIL-2 gene did not show specific binding sites at low or moderate concentrations of added HMG-I, even though this region contains several long stretches of A+T-rich sequence.

Fig. 3*B* summarizes the specific HMG-I-binding sites found in the bIL-2 cDNA sequence. Solid underlines indicate the sites of specific HMG-I binding found in the 3′-UTR. Broken underlines in the 5′ protein-encoding end of the sequence indicate sites that did not specifically bind the HMG-I protein but that might have been expected to do so (29) if short stretches of A+T-rich sequences alone were the sole factor determining such associations.

DISCUSSION

In this report we present evidence for multiple functions of the 3′-UTR of the bIL-2 cDNA. Three different, but possibly related, activities have been demonstrated for this 3′-UTR: (*i*) ability of this sequence ligated to a "marker" (e.g., CAT) gene to regulate the functional activity of the fusion gene inside lymphoid cells *in vivo* under appropriate conditions of experimental induction (Fig. 1); (*ii*) ability of this sequence to confer instability on cognate mRNA transcripts and on heterologous mRNAs into which it is inserted, probably by acting as a structural recognition signal (unpublished data) for a short-lived nuclease(s) (Fig. 2); and (*iii*) ability of the highly conserved A+T-rich sequences located in the 3′-UTR to act as specific binding sites for the nuclear nonhistone HMG-I protein *in vitro* (Fig. 3). Together these results suggest a complex, multifaceted, functional role for the 3′-UTR of the bIL-2 gene *in vivo*.

Regulation of IL-2 production *in vivo* by activated T-helper lymphocytes appears to be controlled at both the transcriptional and posttranscriptional levels (6–8, 13). Activation of lymphocytes by mitogens, phorbol esters, or both greatly increases the levels of IL-2 mRNA as a result of *de novo* gene transcription (6, 30). Concomitant with lymphocyte activation and induction of IL-2 mRNA transcription, chromatin structural changes also occur in the 5′ upstream promoter and enhancer (10) regions flanking the IL-2 gene (31). Subsequent to the induction phase, accumulation of IL-2 mRNA is promptly "downregulated" or shut off (7). This downregulation requires the synthesis of a short-lived "protein repressor" whose production can be prevented by CHX and other inhibitors of protein synthesis (7, 8). Inhibition of this labile "repressor" by treatment with CHX leads to a superinduction of IL-2 mRNA and lymphokine production by lymphocytes (7). From the results reported here it seems likely that



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         9         18         27         36         45         54
GGGCTATCT GTTCGGTCG TTCATGTCA GCAATGTAC AAGATACAA CTCTTGTCT
                             |--> 5'-Coding
        63         72         81         90         99        108
TGCATTGCA CTAACTCTT GCACTCGTT GCAAACGGT GCACCTACT TCAAGCTCT

       117        126        135        144        153        162
ACGGGGAAC ACAATGAAA GAAGTGAAG TCATTGCTG CTGGATTTA CAGTTGCTT

       171        180        189        198        207        216
TTGGAGAAA GTTAAAAAT CCTGAGAAC CTCAAGCTC TCCAGGATG CATACATTT

       225        234        243        252        261        270
GACTTTTAC GCGCCCAAG GTTAACGCT ACAGAATTG AAACATCTT AAGTGTTTA

       279        288        297        306        315        324
CTAGAAGAA CTCAAACTT CTAGAGGAA GTGCTAAAT TTAGCTCCA AGCAAAAAC

       333        342        351        360        369        378
TTGAACCCC AGAGAGATC AAGGATTCA ATGGACAAT ATCAAGAGA ATCGTTTTG

       387        396        405        414        423        432
GAACTACAG GGATCTGAA ACAAGATTC ACATGTGAA TATGATGAT GCAACAGTA

       441        450        459        468        477        486
AACGCTGTA GAATTTCTG AACAAATGG ATTACCTTT TGTCAAAGC ATCTACTCA
           Bcl I
       495 ↓  504        513        522        531        540
ACAATGACT|TGATCACTA AGTGCCTCT CATTTTAAA CTATCAGGC TTTCTATTT
         |--> 3'-UTR
       549        558        567        576        585        594
ATTTAAATA TTTAAAATT TATATTTAT TTTTTGATA TATGTTTTC CTACCTTTT

       603        612        621        630        639        648
GTAACTGTT AGTCTTAAG ATGATAAAT ATGGATCTT TTAAGATTC TTTTTGGTA

       657        666        675        684        693        702
ACTACGGGC TCTAAAAAA TTCAGTTAA ATTATTTAT CCTGAAGTA TTTATTTGT

       711        720        729        738        747        756
ATATTCAAT TTTTAAATA TAATGTCTA TGCAGGTCA TTGACTAAA ATTATTTAA

       765        774        783
TAAAGTTGA TGAATAAAA ACAAAAAAA AAAAAAAA
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FIG. 3. DNase footprint analysis of specific HMG-I-binding sites in bIL-2 cDNA. (*A*) DNase I footprint localization of specific HMG-I binding in the 295-bp 3′-UTR fragment. Lanes 1–4, Sanger dideoxy sequencing lanes (C, T, A, G) corresponding to the 3′-UTR footprinted region. Lanes 5 and 10 show control DNase I digestion patterns in the absence of HMG-I. Lanes 6–9 show the DNase digestion patterns in the presence of increasing amounts of HMG-I (25, 50, 75, and 100 ng). (*B*) Nucleotide sequence of bIL-2 cDNA, showing major sites of observed (solid underlines) and potential but not observed (broken underlines) specific binding of HMG-I.

this previously postulated protein repressor (7, 8) is probably a labile nuclease(s) (Fig. 2). The biochemical nature of this nuclease(s) is not yet known, but preliminary results suggest

(data not shown) that it may be a different enzyme from the well-characterized RNase L activated in interferon-treated cells exposed to double-stranded RNA molecules (4, 32).

Posttranscriptional regulation of gene activity by control of mRNA stability, perhaps by specific nucleases, may be a common mechanism operating for many of those lymphokines, cytokines, and protooncogenes known to be transiently expressed in lymphocytes activated by various stimuli (30, 33, 34). Many of these transiently expressed genes are known to contain long stretches of the conserved $(TATT)_n$ motif (with limited sequence divergence) in their 3'-UTR noncoding domains (13, 14) and, likewise, often display superinduction when activated cells are exposed to protein synthesis inhibitors such as CHX (2, 5, 7, 34). Compilations of some of the numerous immune-induced genes of mammals containing highly conserved A+T-rich sequences in their 3' noncoding regions have been made (13–15). Additional support for the involvement of conserved A+T-rich sequences in conferring instability on mRNAs in mammalian cells comes from the studies of human granulocyte/macrophage colony-stimulating factor (GM-CSF) mRNA by Shaw and Kamen (15), who demonstrated that the short half-life of GM-CSF *in vivo* (<30 min) is due to a long A+T-rich region in the 3'-UTR of this lymphokine gene. And, finally, it has been reported that the 3'-UTR of the c-*fos* protooncogene may control the stability of c-*fos* mRNA *in vivo* in mouse cells (35) and that deletion of an A+T-rich sequence from the 3'-UTR of c-*fos* converts the normally nontransforming gene into a transforming gene (36).

The possible biological role that the nonhistone protein HMG-I might play in the *in vivo* structure or function of the bIL-2 gene is currently unknown. However, the specific binding of this protein to the A+T-rich regulatory sequences in the 3'-UTR (but not the 5' coding region) of the bIL-2 cDNA suggests that it might function in some structural or compartmentational capacity at the level of chromosomal gene organization in the nucleus. Furthermore, since HMG-I appears to be present in appreciable concentrations only in nuclei isolated from rapidly proliferating cells (23, 37), this protein may play some role in the chromosomal organizational changes occurring during the cellular mitotic cycle (38, 39).

As noted previously, the 3'-UTRs of many of the known lymphokine, cytokine, and protooncogenes contain long stretches of conserved $(TATT)_n$ sequence (13). A plausible explanation for such a high degree of evolutionary conservation of specific A+T-rich sequences is that these motifs may perform different functional biological roles depending on whether the nucleotide sequence is located in chromosomal DNA or in mRNA transcripts. For example, it seems likely that at the DNA level these motifs might serve as the recognition sites for binding of specific nonhistone proteins, such as HMG-I, that may be involved with chromosomal structural organization of genes in the nucleus, while at the mRNA level the cognate motifs might serve as recognition signals for nucleases that regulate the posttranscriptional expression of these genes by selective message degradation. Thus, alterations or mutational changes in these A+T-rich sequences that are too drastic might be expected to be selected against, since they could adversely affect several different biological functions at the same time. Such conserved sequences could, additionally, be utilized by lymphoid cells as a mechanism to coordinate the expression and activity of a number of different genes during induction of the immune response.

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