Induction by Torsional Stress of an Altered DNA Conformation 5′ Upstream of the Gene for a High Mobility Group Protein from Trout and Specific Binding to Flanking Sequences by the Gene Product HMG-T†

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ABSTRACT: We have used enzymic and chemical probes to search for altered DNA conformations in the 5′ flanking region of the gene for a high mobility group protein (HMG-T) from trout. This search was conducted in order to identify potential genetic elements that might be involved in the transcriptional control of the HMG-T gene. We identified, in supercoiled plasmid DNA molecules containing a 900 base pair insert of the 5′ region of the gene, an S1-sensitive site situated within an $(AT)_{12}$ sequence approximately 120 base pairs upstream from the start of the HMG-T gene. Chemical modification of supercoiled DNA with the single-strand-selective reagent bromoacetaldehyde was limited to a region coincident with the S1 nuclease site. T7 endonuclease I, a probe highly specific for four-way helical junctions, cleaved predominantly at the boundaries of the $(AT)_{12}$ stretch. These data are most consistent with the interpretation that the $(AT)_{12}$ sequence adopts a cruciform structure when torsionally stressed by negative supercoiling. DNase I footprinting analyses demonstrated that HMG-T protects two regions almost equidistant from the center of the $(AT)_{12}$ sequence, indicating that HMG-T is a sequence-specific DNA binding protein.

Negative supercoiling of DNA is a widespread phenomenon among DNA viruses and plasmids (Menissier et al., 1982; Hamilton et al., 1982; Sunter et al., 1984; Eason & Vinograd, 1971; Wang, 1969; Deleys & Jackson, 1976). Torsional stress induced by negative supercoiling of closed circular DNA has been shown to induce a variety of DNA sequences to undergo conformational transitions [see Lilley (1980) and Greaves et al. (1985) and references cited therein]. The question of whether chromatin domains in eucaryotes, such as the radial loops seen when chromosomes are depleted of histones (Laemmli et al., 1977), can also exist under torsional stress is under active investigation. Experiments with the SV40 minichromosome (Luchnik et al., 1982) and somatic 5S genes injected into *Xenopus* oocytes (Ryoji & Worcel, 1984) have suggested that supercoiled "dyanmic chromatin" can exist and that its formation is ATP and Mg^{2+} dependent (Glikin et al., 1984); moreover, the presence of chromatin in this "dynamic state" correlates well with the ability of the genes within it to be transcribed (Luchnik et al., 1982; Ryoji & Worcel, 1984; Harland et al., 1983).

In the experiments reported here, we show that the torsional stress produced by negatively supercoiling a plasmid containing the gene for a eucaryotic, chromosomal protein of the high mobility group protein class, the trout nuclear protein, HMG-T[1] (Dixon, 1982; Watson et al., 1977), can induce an altered DNA conformation with properties most consistent with those of a cruciform at a specific AT-rich sequence 5′ upstream of the gene. Moreover, sequences flanking this domain provide specific binding sites, as judged by a footprinting analysis, for the product of the gene, the protein HMG-T.

Experimental Procedures

Nucleic Acids. Plasmid pJW11 is a recombinant plasmid derived by cloning into pBR322 a *Bam*HI, *Hin*dIII fragment of 900 base pairs containing the 5′ flanking region and part of the first exon of the HMG-T gene (Figure 1). Supercoiled plasmid DNA was prepared by following the procedure of Greaves et al. (1985) in which the DNA was never subjected to extremes of temperature and pH, nor extracted with phenol. Templates for DNase I footprinting were end-labeled with [γ-^{32}P]ATP by T4 polynucleotide kinase (Maxam & Gilbert, 1980).

S1 Nuclease Assay. DNA was incubated at 100 μg/mL in 50 mM sodium acetate (pH 4.6)/50 mM sodium chloride/1 mM $ZnCl_2$ with 200 units/mL nuclease S1 (Pharmacia) at 15 °C for 2 h. The reaction was terminated by addition of excess Tris-HCl, pH 8.0, and EDTA followed by phenol extraction.

Bromoacetaldehyde. Bromoacetaldehyde was prepared by acid hydrolysis of bromoacetaldehyde diethyl acetal and vacuum distillation. Reactions were routinely performed at 37 °C for 30 min in 50 mM sodium acetate, pH 4.5, containing 100 mM bromoacetaldehyde and 100 μg/mL plasmid DNA as previously described (Lilley, 1983). No qualitative differences in DNA modification sites were observed when reactions were performed at neutral pH in 10 mM Tris-HCl.

T7 Endonuclease I. T7 gene 3 product endonuclease I (de Massy et al., 1984) was a gift of Dr. Paul Sadowski. DNA at 100 μg/mL was incubated in 50 mM Tris-HCl (pH 8.0)/3 mM $MgCl_2$/5 mM β-mercaptoethanol with approximately 50 units/mL T7 endonuclease I for 30 min at room temperature.

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[1] Abbreviations: bp, base pair(s); kb, kilobase(s); HMG, high mobility group; EDTA, ethylenediaminetetraacetic acid; Tris, tris(hydroxymethyl)aminomethane; Hepes, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; kDa, kilodalton(s); HPLC, high-performance liquid chromatography.

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FIGURE 1: Map of restriction endonuclease sites found within the 5′ flanking region (thin line) and part of the first exon (thick line) of the HMG-T gene. Symbols: B, *Bam*HI; Hd, *Hin*dIII; He, *Hae*III; S, *Sau*3A.

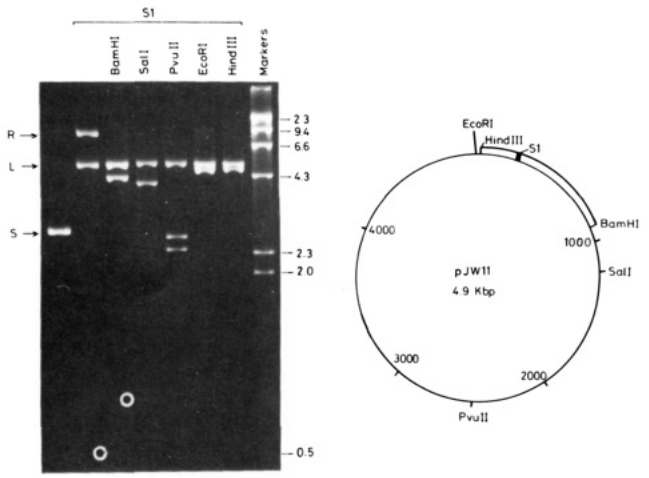

FIGURE 2: Site-specific cleavage of supercoiled pJW11 by S1 nuclease. DNA was incubated with S1 nuclease followed by complete digestion with a restriction endonuclease. The samples were subjected to electrophoresis in 0.8% agarose. Tracks contained (from left to right) untreated supercoiled pJW11, supercoiled pJW11 incubated with S1 nuclease, supercoiled pJW11 incubated with S1 and subsequent digestion to completion with *Bam*HI, *Sal*I, *Pvu*II, *Eco*RI, or *Hin*dIII, respectively, and bacteriophage λ digested with *Hin*dIII to provide DNA markers, the sizes of which (in base pairs) are indicated on the right. The arrows on the left show the migration positions of supercoiled (S), relaxed circular (R), and linear (L) pJW11. A restriction map of pJW11 is shown on the right, with the 5′ upstream region of the HMG-T gene indicated as an open box and the site of S1 cleavage as a closed box. In the *Bam*HI and *Sal*I lanes, white circles have been added to mark the ≃600 and ≃900 bp fragments, respectively, which are quite faint. They are seen more clearly in Figure 3.

DNase I Footprinting. Footprinting reactions were performed in 25 mM Hepes (pH 7.9)/80 mM sodium chloride/5 mM $MgCl_2$/1 mM dithiothreitol/100 μg/mL BSA/25–50 ng of end-labeled DNA and 2 μg of *Escherichia coli* DNA in a total volume of 25 μL (Emmerson & Felsenfeld, 1984). Binding was allowed to proceed for 20 min on ice. Three volumes of 5 mM $CaCl_2$/10 mM $MgCl_2$ and an empirically determined amount of DNase I (Worthington) were added, and digestion was allowed to proceed at room temperature for 30 s. After addition of 50 μL of 1.5 M sodium acetate/20 mM EDTA/100 μg/mL yeast tRNA, the mixture was extracted with phenol and precipitated with ethanol.

Gel Electrophoresis. DNA samples were fractionated by electrophoresis in 0.8% agarose gels (Sharp et al., 1973) or 6% acrylamide (Maniatis et al., 1975). DNA samples were extracted from low-gelling-temperature agarose (Sigma) by heating to 68 °C and extracting twice with phenol and once with chloroform.

RESULTS

S1-Sensitive Site Upstream of the HMG-T Gene. Figure 2 shows the results of cleavage of supercoiled pJW11 plasmid by nuclease S1. When the supercoiled DNA (S) was treated with S1 nuclease, it was initially converted to nicked circles (R) and then to the linear form (L). Subsequent digestion

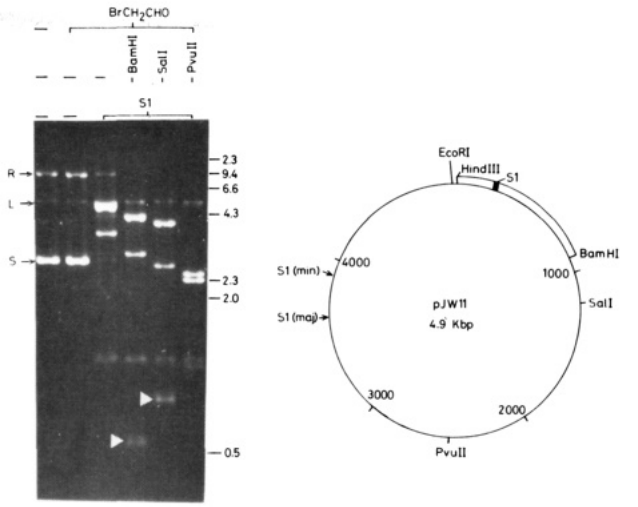

FIGURE 3: Site-selective modification of supercoiled pJW11 by bromoacetaldehyde. Supercoiled DNA was incubated with bromoacetaldehyde ($BrCH_2CHO$) followed by complete digestion by *Bam*HI, *Sal*I, or *Pvu*II and finally incubated with S1 nuclease to cleave modified sites. Samples were subjected to electrophoresis in 0.8% agarose. Tracks contained (from left to right) untreated supercoiled pJW11, supercoiled pJW11 treated with $BrCH_2CHO$, supercoiled pJW11 treated with $BrCH_2CHO$ and then cleaved with S1 nucleases, and supercoiled pJW11 treated with $BrCH_2CHO$, subsequently digested with *Bam*HI, *Sal*I, or *Pvu*II, respectively, and finally incubated with S1 nuclease. Bacteriophage λ digested with *Hin*dIII as size markers (in base pairs) is shown on the right. A restriction map of plasmid pJW11, with the sites modified by $BrCH_2CHO$ in the 5′ upstream region of the HMG-T gene (S1) and pBR322 [S1 (min) and S1 (maj)] indicated, is shown on the right. White arrowheads mark the ≃600 and ≃900 bp bands in the *Bam*HI and *Sal*I lanes.

of S1-treated pJW11 with restriction endonucleases which cleave unique sites within pBR322 generated a pattern of discrete bands. From the known position of the restriction sites, it can be deduced that cleavage by S1 occurred at a specific sequence located 120–140 base pairs upstream from the start of the HMG-T gene. The specificity for this site is maintained at temperatures betwen 4 and 37 °C. Moreover, an absolute requirement for supercoiling was shown for the structural perturbation to be present in the DNA by the lack of S1 cleavage of linear pJW11 (data not shown).

Bromoacetaldehyde Modifies the S1-Sensitive Site. The possibility that S1 nuclease induced the structural perturbations in the supercoiled DNA was considered. We, therefore, probed pJW11 DNA with bromoacetaldehyde, a chemical reagent which modifies single-strand DNA at adenine residues (Lilley, 1983). Following the bromoacetaldehyde reaction, the DNA was cleaved with restriction endonuclease to relax supercoils, and the modified sites in the DNA were cleaved with S1 nuclease. Figure 3 shows that this treatment of the DNA generated a pattern of predominant bands identical with those produced by the S1 cleavage experiments, indicating that the bromoacetaldehyde modified a region of the DNA coincident with the S1 cleavage site. Additional fainter bands are also observed in Figure 3. The production of these fainter bands is consistent with simultaneous modification of the site within the 5′ flanking region of the HMG-T gene and two sites within pBR322. The sites within the vector correspond to the major and minor S1-sensitive sites at positions 3059 and 3229 base pairs, respectively, in the pBR322 sequence (Lilley, 1980). Thus, the faint bands in the 1.5-kb region represent S1 cutting at the major and minor sites in pBR322 as well as at the site 120–140 bp upstream from the HMG-T start site in the insert. We offer two possible explanations for this result. In supercoiled pJW11, an equilibrium may exist between the altered

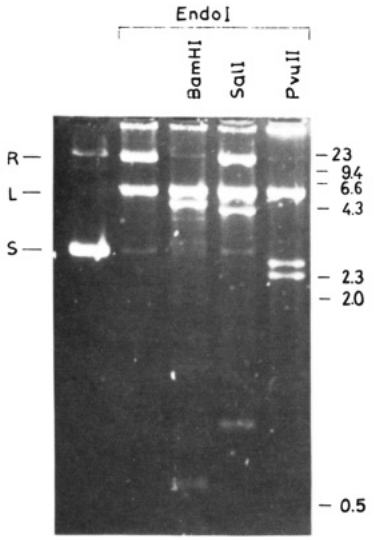

FIGURE 4: Site-specific cleavage of supercoiled pJW11 with T7 endonuclease I. DNA was incubated with T7 endonuclease I followed by complete digestion with *Bam*HI, *Sal*I, or *Pvu*II. The samples were subjected to electrophoresis in 0.8% agarose in the order (from left to right) untreated supercoiled pJW11, supercoiled pJW11 incubated with T7 endonuclease I, and supercoiled pJW11 incubated with T7 endonuclease I followed by complete digestion with *Bam*HI, *Sal*I, or *Pvu*II, respectively. Molecular weight markers in base pairs are shown on the right.

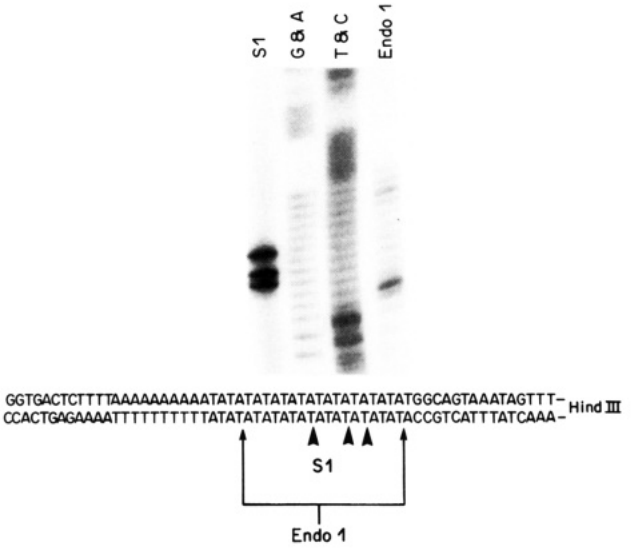

FIGURE 5: Phosphodiester bonds cleaved in the 5′ upstream region of the HMG-T gene by S1 nuclease and T7 endonuclease I. Supercoiled pJW11 was incubated with either S1 nuclease or T7 endonuclease I, the products were subjected to electrophoresis in low-gelling-temperature agarose, and the nicked form of the plasmid was isolated as described under Experimental Procedures. Following complete cleavage of the DNA with *Hin*dIII, the noncoding strand was radioactively labeled at the 5′ end with [γ-^{32}P]ATP. The samples were subjected to electrophoresis on a 6% (w/v) polyacrylamide sequencing gel with purine (G and A) or pyrimidine (T and C) DNA sequencing markers derived by formic acid or hydrazine modification, respectively, of the 5′-^{32}P-labeled 900 bp *Bam*HI–*Hin*dIII fragment from pJW11. The autoradiograph of the gel is shown above with the assignment of the cleaved phosphodiester bonds by S1 nuclease (S1) and T7 endonuclease I (Endo I) shown below.

conformation recognized by S1 nuclease in the HMG-T gene and the major and minor S1 sites within pBR322, with the former predominating. The lack of recognition by S1 nuclease of the sites within the vector is probably due to the fact that the supercoiled molecule is rapidly converted to a nicked species, thereby releasing the torsional stress driving the formation of the altered DNA conformation. Since modification of the DNA by bromoacetaldehyde did not, by itself, abolish the superhelical density of the molecule (see Figure 3, second lane from left), sufficient opportunity for modification of the other sites located in pBR322 would be available. The alternative explanation for the result is that modification by bromoacetaldehyde of the site within the HMG-T gene may drive the equilibrium toward the altered conformations within the vector. No qualitative difference was seen in the pattern of bands generated when the bromoacetaldehyde reaction was conducted at pH 7.0 (data not shown). Thus, specificity for this site was maintained at pHs between 4.5 and 7.0.

The Holliday Resolvase T7 Endonuclease I Cleaves at the Site of the Altered DNA Conformation. The structural perturbation located in the 5′ flanking region of the HMG-T gene, recognized by S1 nuclease and bromoacetaldehyde, must generate a localized single-stranded region in supercoiled DNA. A variety of conformational transitions such as base unpairing, Z-DNA, and cruciform and slippage structures induced by negative supercoiling can create such localized regions of single-stranded DNA. We used T7 endonuclease I, an enzyme that cleaves specifically sites located in the stem–base region of a cruciform (de Massy et al., 1984), to probe the altered conformation in the 5′ flanking region of the HMG-T gene. We found that, as with S1 nuclease and bromoacetaldehyde, T7 endonuclease I cleaved within a region of the supercoiled DNA approximately 120 base pairs upstream from the start of the HMG-T gene, indicating that the altered DNA conformation is most probably a cruciform (Figure 4).

Fine Mapping of S1 Nuclease and T7 Endonuclease I Cleavage Sites. We mapped the phosphodiester bonds cleaved by S1 nuclease and T7 endonuclease I within the 5′ flanking region of the HMG-T gene. Supercoiled pJW11 was digested with either S1 nuclease or T7 endonuclease I, and the products were fractionated by electrophoresis in low-gelling-termperature agarose. The cleaved form of the plasmid was extracted from the gel and digested with *Hin*dIII. The DNA was 5′ end-labeled and cut with a second restriction enzyme (i.e., *Bam*HI), and the sequence of interest was isolated. The fragment was fractionated on a 6% polyacrylamide–urea gel alongside Maxam and Gilbert DNA sequence markers (Maxam & Gilbert, 1980). Figure 5 shows that S1 cleaved three phosphodiester bonds on the bottom strand (see the DNA sequence depicted in Figure 5) within an $(AT)_{12}$ sequence. Two major cleavage sites, located at the boundaries of the $(AT)_{12}$ sequence, were recognized by T7 endonuclease I. Additional phosphodiester bonds throughout the $(AT)_{12}$ sequence were also cleaved but less frequently by T7 endonuclease I. This pattern of cleavage by T7 endonuclease I suggests varying degrees of cruciform extrusion (or "breathing") in supercoiled molecules.

Although the pattern of cleavage of T7 endonuclease I and S1 nuclease at the $(AT)_{12}$ sequence is not absolute proof that the structural perturbation is a cruciform, the properties of the altered conformation are most consistent with those of a cruciform. Others have also shown that long stretches of alternating dA-dT contained in plasmids extrude to form cruciforms in response to negative supercoiling rather than undergo other conformational transitions (Greaves et al., 1985; Haniford & Pulleyblank, 1985; Panyutin et al., 1985).

HMG-T Binds a Sequence in Its Own Gene. The finding of a structural perturbation in the 5′ flanking region of the HMG-T gene suggested to us that this region might act as a binding site for trans-acting regulatory factors. We explored this notion by conducting DNase I footprinting analyses of the upstream region of the HMG-T gene using protein extracts

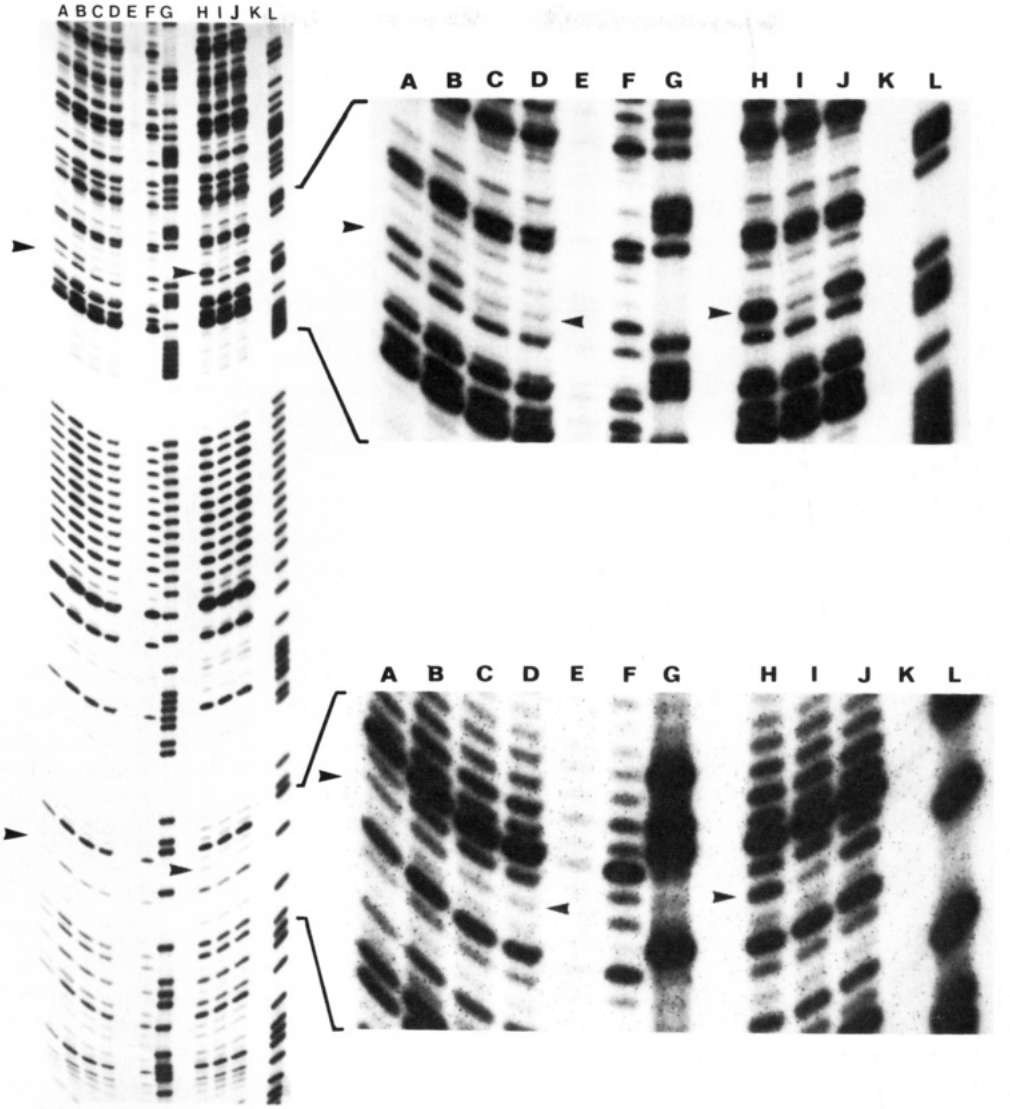

FIGURE 6: DNase I footprint analysis of the HMG-T protein on the 5′ upstream region of its own gene. The 900 bp *Hin*dII–*Bam*HI fragment of pJW11 5′-^{32}P labeled at the *Hin*dIII end was subjected to DNase I footprinting analysis as described under Experimental Procedures. The products of this analysis were subjected to electrophoresis in a 6% polyacrylamide sequencing gel. (A) Control; (B) 0.2 μg of HMG-T; (C) 1.0 μg of HMG-T; (D) 2.0 μg of HMG-T; (E) 5.0 μ of HMG-T; (F) control; (G) Maxam–Gilbert purine sequencing markers; (H) control; (I) 2.0 μg of HMG-T; (J) 2.0 μg of H6; (K) 2.0 μg of H1/H5; (L) Maxam–Gilbert purine sequencing markers. The panel on the left shows the footprint generated by HMG-T (arrowed) flanking the $(AT)_{12}$ sequence. Enlarged photographs of the footprinted regions are shown on the right.

from salt washes of trout testis nuclei. Preliminary results indicated that a factor enriched in a 0.5 M salt extract, but not detected in a 0.25 M salt extract of trout nuclei, generated a footprint in two regions flanking the $(AT)_{12}$ sequence. This result prompted us to test proteins found in the 0.5 M salt wash that had already been purified and characterized (Dixon, 1982; Watson et al., 1977; McLeod et al., 1977) for their ability to develop a footprint on the HMG-T gene. These included purified preparations of the high mobility group proteins from trout nuclei, H6 and HMG-T, and a mixture of histones H1 and H5 isolated from trout erythrocytes. No footprint was generated by H6, whereas the histone H1/H5 totally abolished nicking of the DNA by DNase I (Figure 6). However, HMG-T protected two regions on the noncoding strand from DNase I digestion located almost equidistant fom the center of the $(AT)_{12}$ sequence. The footprint was restricted to protection of one or two thymidine residues residing in the sequences TTCA and ACTT at positions −171 to −168 and −85 to −89, respectively (Figure 7). No footprint was generated by HMG-T on an isolated (nonsupercoiled) 260 base pair *Hae*III–*Sau*3A fragment spanning the $(AT)_{12}$ sequence and

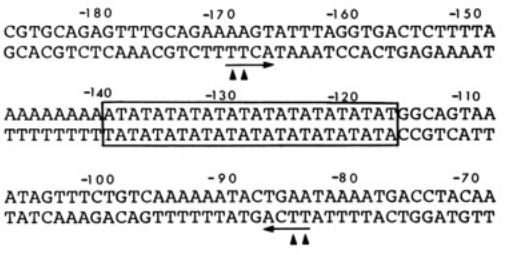

FIGURE 7: Nucleotide sequence of the 5′ upstream region of the HMG-T gene showing the $(AT)_{12}$ stretch that adopts a cruciform conformation when torsionally stressed by negative supercoiling (boxed sequence), the protected region in DNase I footprinting experiments (arrowheads), and the TTCA/ACTT sequence (underlined). The base pair numbering represents the distance upstream of the transcription start.

radioactively labeled at the 5′ end of the coding strand. Calf thymus HMG-1 developed an identical footprint as with HMG-T (data not shown).

A hierarchical DNA binding activity for HMG-T was observed in the footprinting experiments. At low concentrations of HMG-T, sequence-specific protection from DNase I di-

gestion was observed. At higher concentrations of HMG-T, DNase I digestion was almost completely abolished, indicating a random binding of HMG-T to the DNA similar to the binding of histones H1/H5.

Discussion

The high mobility group (HMG) proteins are abundant, widely distributed chromosomal proteins. Although the biological functions of the HMG proteins have not been clearly established, they have been implicated in control of transcription and replication (Dixon, 1982; Goodwin & Matthew, 1982). The large HMG proteins, such as HMG-1 and -2 from calf thymus and HMG-T from trout, bind equally well to procaryotic and eucaryotic DNA, suggesting that binding is independent of nucleotide sequence (Shooter et al., 1974; Goodwin et al., 1975). However, recent studies have shown preferential binding of HMG-1 and -2 to single-strand regions, especially those located in B–Z junctions and cruciforms induced by the torsional stress of supercoiling (Isaackson et al., 1979; Hamada & Bustin, 1985). In addition, Brown and Anderson (1986) reported the hierarchical binding of chicken HMG-2a to synthetic polynucleotides in the order (dI·dC) > (dA-dT)·(dA-dT) > (dA)·(dT) ≫ (dG)·(dC) > (dG·dC)·(dG-dC), suggesting interaction of HMG proteins with sequences of reduced stability at physiological temperatures and ionic strengths.

A recent report from our laboratory has shown that covalent photo-cross-linking of HMG-T to DNA sequences in trout liver, followed by selective retrieval of the protein–DNA adducts with specific antibodies, greatly enriched DNA sequences in proximity to a subset of the family of protamine genes, but not the histone and vitellogenin genes (Blanco et al., 1985).

We have demonstrated here that the HMG-T protein binds selectively to two sequence 5′ upstream of its own gene. These sequences flank a domain that adopts an altered conformation, exhibiting properties most consistent with those of a cruciform, in response to negative supercoiling. The binding of HMG-T to the sequence TTCA-26 bp-cruciform $(TA)_{12}$-29 bp-ACTT on only one strand is atypical of DNA binding proteins examined thus far (Figure 7). The distance of 87 bp between the two protected regions in the DNase I footprint suggests that, at least, two molecules of HMG-T are involved in binding. The TTCA/ACTT motif may act as the binding site to position the two HMG-T proteins in the correct orientation. It is important to note, however, that the TTCA/ACTT motifs do not constitute inverted repeats since both are on the *same* strand of the DNA. This would imply that the HMG-T molecule binds to the TTCA and ACTT motifs independent of their polarity and would further suggest that the interaction with the sequence of bases themselves rather than the phosphate–sugar backbone determines the interaction. The binding of HMG-T to its own gene could be of considerable biological significance. However, it is clear that the amount of the 29-kDa HMG proteins is far higher than required to bind to a subset of single-copy genes. A recent report (Tremethick & Molloy, 1986) has suggested, in fact, that HMG-1 may have a generalized positive transcription factor activity. Thus, it is envisaged that many sites of the type seen here upstream of the HMG-T gene and in the vicinity of a subset of the trout protamine genes (Blanco et al., 1985) may exist in the genome.

We have conducted a survey of the literature for other genes exhibiting sequence homology in potential regulatory regions to the HMG-T binding domain in the HMG-T gene. Of particular interest is a sequence 540 bp upstream from the human β-globin gene which exists in an altered conformation in supercoiled DNA and is protected from S1 nuclease digestion by HMG-1 and -2 (Cockerill & Goodwin, 1983). Moreover, footprint analysis of a mature erythrocyte protein fraction selectively protected this region from DNase I cleavage (Plumb et al., 1985). We found, however, that purified HMG-T developed a footprint 180–261 bp downstream from this S1-sensitive site in the human β-globin gene (unpublished observation). As with the HMG-T gene, the protected regions of one or two thymidine residues reside in an ACTT motif.

Recently, Solomon et al. (1986) have recently reported DNase I footprinting experiments which show that a high mobility group protein from African green monkey, the α-protein (or HMG-I), binds with equal affinity to any run of 5 or 6 A·T bp in duplex DNA. Interaction of α-protein with DNA occurs by extensive contacts with the minor grove of the DNA (Solomon et al., 1986). Elton et al. (1986) have confirmed the binding of HMG-I to A-T-rich DNA regions using HMG-I purified by reverse-phase HPLC.

The limited footprint generated by α-protein on stretches of 5 or 6 A·T bp shows similarity to the footprint (or toe-print) generated by the HMG-T protein on its own gene; however, significant differences are apparent. Whereas the α-protein protects from DNase I digestion both strands of the DNA in a stretch of 5 or 6 A·T bp, the HMG-T protein protects only one strand of the DNA duplex within a TTCA/ACTT motif. However, the TTCA/ACTT sequence in the HMG-T binding site is embedded in an A·T-rich milieu, so that it is particularly interesting that many of the α-protein binding sites in the 172 bp repeat of the α-satellite DNA SV40 also contain the TTCA/ACTT motif within, or immediately juxtaposed to, the protected sequence (Solomon et al., 1986) Thus, the HMG-T binding sites in DNA may represent a subset of the α-protein binding sites.

Also of significance is the lack of HMG-T binding, as judged by DNase I footprint experiments, to runs of 5 or more A·T bp within the 5′ upstream region of the HMG-T and human β-globin genes. If binding of HMG-T to A·T-rich sequences were facilitated by particular aspects of the minor grove, as is the case for α-protein, the lack of HMG-T binding to certain runs of A·T base pairs [for example, the $A_9(AT)_{12}$ sequence that adopts an altered conformation when torsionally stressed] may be due to flanking sequences, or to the A·T-rich sequence itself, influencing the conformation of the minor or major groove of B-DNA.

It is important to note the differences in the properties of the α-protein and HMG-T protein. The α-protein is a ~10-kDa protein that preferentially binds double-stranded DNA (Solomon et al., 1986), whereas the large HMG proteins of calf thymus (HMG-1 and -2) (Walker, 1982) and of trout (HMG-T) (Dixon, 1982) are ~29-kDa proteins which preferentially bind single-strand DNA (Isaackson et al., 1979). Clearly, the α-protein and the large HMG proteins of calf thymus and trout represent two distinct classes of the family of HMG proteins. The apparent differences in the DNA binding properties of the α-protein and HMG-T proteins might reflect different functional properties.

It will be important to determine whether the HMG-T protein is bound in vivo to its own gene, and the relationship of the putative cruciform structure induced by the torsional stress of negative supercoiling to the binding, in order to ascertain whether the specific binding sites for HMG-T on naked DNA are retained or blocked in chromatin.

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