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High mobility group proteins 1 and 2 stimulate binding of a specific transcription factor to the adenovirus major late promoter

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ABSTRACT

High mobility group proteins 1 and 2 (HMGs 1 and 2) are abundant chromosomal proteins which are believed to be preferentially associated with regions of active chromatin. Our previous results have shown that HMGs 1 and 2 can significantly stimulate specific transcription in vitro from the adenovirus major late promoter. This stimulation is now shown to be due, at least in part, to the influence of HMGs 1 and 2 on binding of a specific transcription factor (MLTF) upstream of the start site of the gene to a region (-66 to -51) which is required for optimal transcription both in vivo and in vitro. HMGs 1 and 2 cause both an increase in the rate of binding of the transcription factor to the DNA and alterations to the pattern of the DNaseI footprint of the factor on the DNA. Different binding states of the factor are also observed dependent on the presence of $MgCl_2$, the factor being bound but not protecting the binding region from DNaseI in the absence of $MgCl_2$.

INTRODUCTION

High mobility group proteins 1 and 2 (HMGs 1 and 2) are abundant chromosomal proteins (Mr ≈ 26 to 29 KD) which appear to be preferentially associated with active regions of chromatin (for review, see ref. 1). This has been inferred from their preferential release upon mild digestion of chromatin with either DNaseI or micrococcal nuclease (2-5). The addition of HMGs 1 and 2 has also been shown to restore the micrococcal nuclease sensitivity of chromatin depleted of histone H1 and non-histone proteins (6). As the amount of HMGs 1 and 2 present in HeLa whole cell transcription lysate is very low, it has been possible to show using an in vitro transcription system that HMGs 1 and 2 can stimulate specific transcription by both RNA polymerases II and III, and can overcome the inhibitory effects of histones (7).

The RNA polymerase II promoter used in these in vitro studies was the adenovirus major late promoter. Work in a number of laboratories has demonstrated that a specific transcription factor (MLTF or USF) which has

been partially purified from whole-cell or nuclear extracts of uninfected HeLa cells binds to sequences in the region -66 to -51 (upstream promoter element or UPE) of the adenovirus major late promoter (8-13). This region has been shown by deletion and mutation analysis to be essential for optimal expression from the promoter (13-17). The "TATA-binding" protein, TFIID, appears to interact with and stabilize the binding of the transcription factor (11). We here examine specifically the effect of HMGs 1 and 2 on the binding of MLTF to the adenovirus major late promoter.

MATERIALS AND METHODS

DNA fragments

DNA fragments were prepared from the plasmid pML $(C_2AT)_{19}$, the kind gift of M. Sawadogo and R. Roeder (11,18). The S (short) or L (long) fragments were prepared by digestion with AvaII and either HhaI or SmaI respectively (see Figure 1). They were either 3'-end labelled using the Klenow fragment of DNA polymerase I or 5'-end labelled using polynucleotide kinase according to standard methods, and purified by non-denaturing gel electrophoresis (19).

Preparation of MLTF-containing fraction

The fraction AA which contains MLTF was prepared from whole cell extracts of HeLa cells according to Carthew *et al.* (8); i.e. as successive flow through fractions from P11 phosphocellulose and DEAE-sephacel columns. The protein concentration was determined to be 1.25 μg/μl.

Preparation of HMG proteins 1 and 2

Calf thymus was directly extracted using 5% perchloric acid, followed by acetone/HCl precipitation (20). The supernatant containing HMG proteins 1 and 2 was further fractionated essentially by the procedure of Goodwin and Johns (21) except that CM Sephadex C50 was used instead of CM Sephadex C25. HMGs 1 and 2 prepared in this way were estimated to be >90% pure and show similar stimulatory activity as HMGs 1 and 2 prepared by differential trichloracetic acid precipitation (7).

DNA binding assay

The assay and analytical electrophoresis were performed according to Carthew *et al.* (8). Incubations (20 μl) contained 0.3 ng of S fragment or 1.2 ng L fragment DNA and 200 to 500 ng poly (dI-dC).poly (dI-dC) and were done either in the absence of $MgCl_2$ or in the presence of 5 mM $MgCl_2$. When HMGs 1 and 2 were added, they were incubated with DNA for 10 min at 30°

prior to addition of the AA fraction and incubation buffer; the level of HMGs 1 and 2 was 1 μg per reaction unless specified. Quantitation of autoradiographs shown in Figure 4 was performed by densitometry using an LKB Ultroscan 2202 densitometer.

DNaseI footprinting

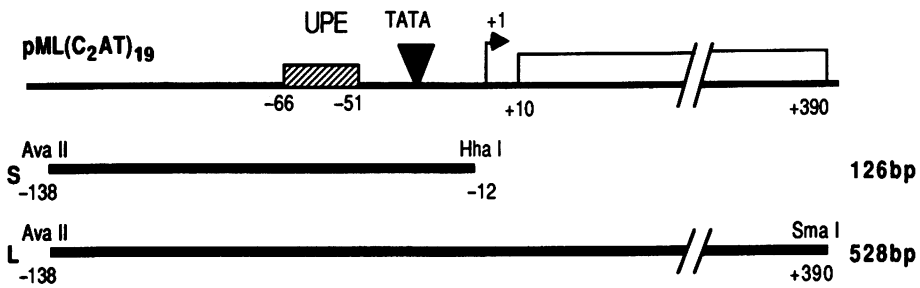
Following incubations of DNA fragments with AA fraction under different conditions (see Figures 5, 6 and 7), 2 μl of suitably diluted DNaseI (Promega, RQ DNase) and $MgCl_2$, where necessary, to adjust the final $[Mg^{2+}]$ to 5 mM were simultaneously added and digestion allowed for to continue for 15 sec. Reactions were stopped by addition of EDTA to 10 mM. They were processed by either (i) treatment with 50 μg/ml proteinase K in the presence of 10 mM EDTA, 0.17% SDS, 0.5 M NH_4CH_3COO and 3 μg of tRNA for 30 min at 45°, followed by phenol extraction and ethanol precipitation, or (ii) incubation mixes were separated on low-ionic strength polyacrylamide gels (8) prior to elution of bands in the above buffer overnight, phenol extraction and ethanol precipitation. The DNA was then analysed on standard 8% acrylamide, 0.4% bisacrylamide, 8 M urea sequencing gels. The adenine plus guanine sequencing reaction was done according to Maxam and Gilbert (22).

RESULTS

Binding of transcription factor, MLTF, to promoter-containing DNAfragments

A HeLa cell lysate fraction (AA) containing MLTF activity was isolated as the successive flow through fractions on phosphocellulose and DEAE-sephacel columns (8). The specific binding activity of the fraction was demonstrated using restriction fragments of the plasmid $pML(C_2AT)_{19}$ which contain the adenovirus major late promoter (Figure 1); two fragments, from the AvaII site at base -138 to either the HhaI site at -12 or the SmaI site at +390, have been used in this study and are referred to as S (short, 126 base pairs) or L (long, 528 base pairs). An equimolar mixture of the two end-labelled fragments, L and S, was incubated with increasing amounts of the MLTF-containing fraction, AA, and the products analysed by the gel retardation method (8). Under standard incubation conditions specific DNA-protein complexes form which can be visualised as the slowly migrating bands, BS and BL, corresponding to the bound short and long fragments respectively (Figure 2). The L fragment has consistently been seen to be bound with a slight preference to the S fragment. When incubations were

A.



B.

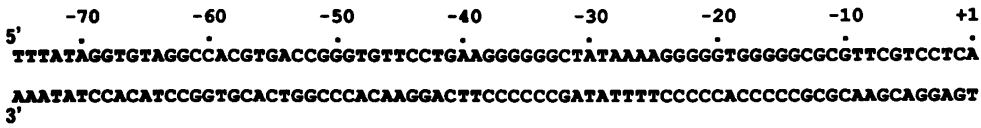


Figure 1. Promoter region of pML(C_2AT)$_{19}$.
A. The plasmid pML(C_2AT)$_{19}$ (Sawadogo and Roeder, 1985a) contains the promoter region (bases -400 to +10) of the adenovirus type 2 major late transcription unit fused to a "G-less" cassette (open box, bases +10 to +390). The TATA box is located at bases -29 to -23 and the upstream promoter element (UPE) at position -66 to -51 is indicated by the hatched box. The restriction fragments between the AvaII site (base -138) and either HhaI site at -12 (S fragment) or the SmaI site at +390 (L fragment) were used in this study.
B. Sequence of the promoter region.

done in the absence of $MgCl_2$ sub-optimal binding of the fragments with MLTF was observed; at a given level of the MLTF-containing fraction, only about 10 to 15% as much fragment was bound as in the presence of $MgCl_2$.

Stimulation of binding of MLTF by HMGs 1 and 2

When HMGs 1 and 2 were added to incubations of the MLTF-containing fraction with promoter-containing fragments in the presence of 5 mM $MgCl_2$, only a slight increase in complex formation was observed (data not shown). Under the standard assay conditions essentially all the MLTF is bound to the promoter-containing DNA fragments during the incubation period. We therefore examined the effect of HMGs 1 and 2 on binding of MLTF to target DNA (S fragment) under sub-optimal conditions in the absence of

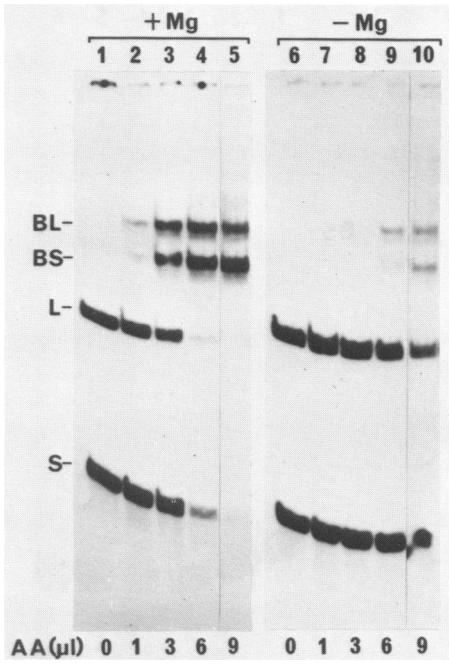


Figure 2. Gel electrophoretic MLTF-DNA binding assay.
The 3' end-labelled L and S DNA fragments (1.5 ng of total DNA in a 1:1 molar ratio of fragments) were incubated for 40 min with 200 ng of poly(dI-dC).poly(dI-dC) and increasing amounts of the AA protein fraction. Unbound DNA was separated from the MLTF-DNA complex by gel-retardation electrophoresis; L and S indicate the positions of the free L and S DNA fragments while BL and BS indicate L and S fragments respectively bound with MLTF. Incubations for tracks 1 to 5 were done in the presence of 5 mM $MgCl_2$ and those in tracks 6 to 10 without $MgCl_2$.

$MgCl_2$. Under these conditions (Figure 3), formation of the specific MLTF-DNA complex is reduced but in the presence of HMGs 1 and 2 the level of binding is substantially increased to levels essentially the same as optimal binding in the presence of $MgCl_2$. Formation of a second complex migrating more slowly than BS is also substantially enhanced in the presence of HMGs 1 and 2. This complex has a similar electrophoretic mobility to Band II described by Carthew et al. (8).

In the presence of HMGs 1 and 2, without AA fraction, no specific band is seen, but only a smearing of the "unbound" DNA fragment at high levels of HMGs 1 and 2 (data not shown). Also, the observed stimulation of MLTF

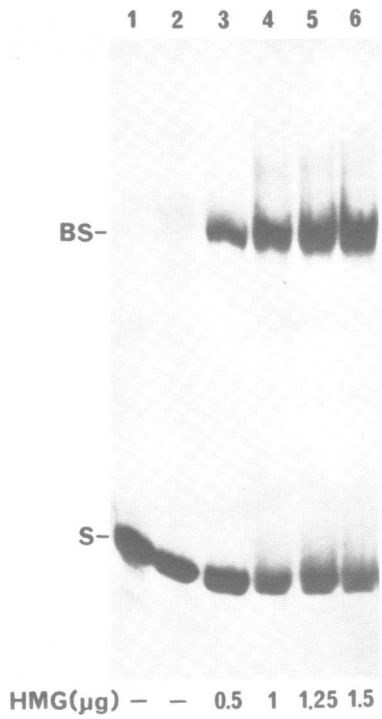


Figure 3. Stimulation of MLTF binding by HMGs 1 and 2.
Incubations were done for 40 min in the absence of $MgCl_2$ using 0.3 ng of 3' end-labelled S fragment, 400 ng poly(dI-dC) and 6 μl (7.5 μg) of AA fraction (except for track 1 in which no AA fraction was present). Incubations in tracks 2 to 6 contained increasing amounts of HMGs 1 and 2, pre-incubated with the DNA fragment for 10 min.

binding is not observed when other proteins are substituted for HMGs 1 and 2; incubations containing equivalent amounts of lysozyme, bovine serum albumin or polyaspartic acid show only small increases in the amount of complexes formed after longer incubations (data not shown)

HMGs 1 and 2 increase the rate of association of MLTF with promoter-containing DNA fragments

In order to determine the mode of action of HMGs 1 and 2 in increasing the binding of MLTF, the kinetics of both binding and dissociation of MLTF and the DNA fragment S were examined. The results of a time-course of MLTF-DNA binding in the absence of $MgCl_2$ are shown in Figure 4A. A difference in rate of binding of approximately 8-fold can be estimated at

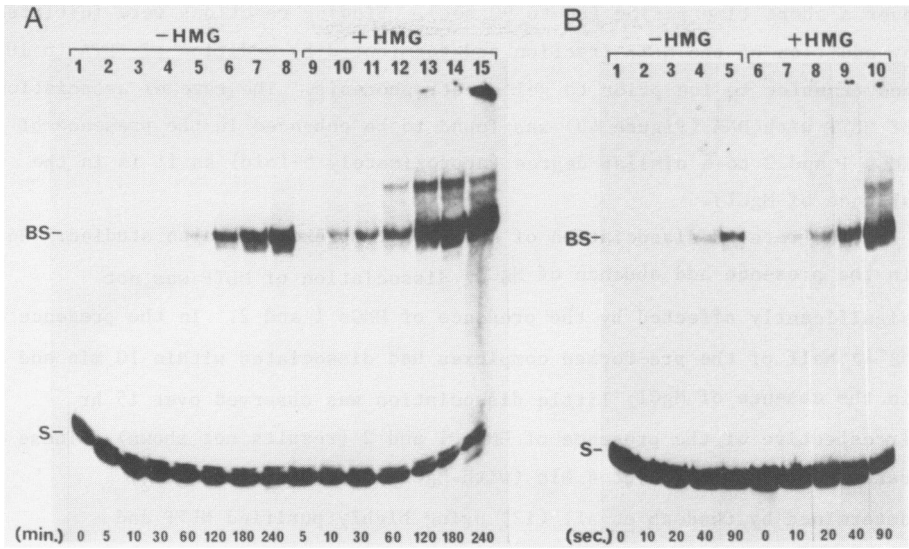


Figure 4. Association rate of MLTF with DNA.

Reactions contained 0.3 ng of 3' end-labelled S fragment and 500 ng of carrier poly(dI-dC).poly(dI-dC): Panel A: DNA was incubated without (tracks 1 to 8) or with (tracks 9 to 15) 1 μg of HMGs 1 and 2 for 10 min at 30° followed by addition of 6 μl of AA fraction and incubation under standard conditions (without $MgCl_2$) for up to 4 hr. For the control (track 1) no AA fraction was added. Incubations were set up at different times to terminate at the same time for analysis by the gel-electrophoresis DNA binding assay. Panel B: DNA was incubated without (tracks 1 to 5) or with (tracks 6 to 10) 1 μg of HMGs 1 and 2 for 10' at 30° followed by addition of 6 μl of AA fraction under standard conditions (with 5 mM $MgCl_2$). Incubations of up to 90 sec were stopped by addition of EDTA to 10 mM and transfer to ice prior to gel-electrophoresis.

times up to 1 hr (e.g. compare 5 and 10 min with 1 μg HMGs 1 and 2 with 30 and 60 min in their absence). It is clear that, both in the presence and absence of HMGs 1 and 2, the time taken to achieve equilibrium binding in the absence of $MgCl_2$ is very long. The lower level of MLTF bound in the absence of $MgCl_2$ (Figure 2) is thus primarily due to a slower rate of association, rather than a difference in the amount of factor which can eventually be bound.

Since under the standard incubation conditions (40 min), in the presence of $MgCl_2$, little difference was observed in MLTF binding with or without HMGs 1 and 2, the rate of association of MLTF with DNA was studied

over a short time period (up to 90 sec). Binding reactions were initiated by addition of the MLTF fraction and terminated by addition of EDTA to 10 mM and transfer to ice prior to gel electrophoresis. The rate of association of MLTF with DNA (Figure 4B) was found to be enhanced in the presence of HMGs 1 and 2 to a similar degree (approximately 5-fold) as it is in the absence of $MgCl_2$.

The rate of dissociation of MLTF-DNA complexes was also studied. Both in the presence and absence of $MgCl_2$ dissociation of MLTF was not significantly affected by the presence of HMGs 1 and 2. In the presence of $MgCl_2$ half of the pre-formed complexes had dissociated within 10 min and in the absence of $MgCl_2$ little dissociation was observed over 15 hr irrespective of the presence of HMGs 1 and 2 (results not shown). These rates compare with about 4 min (with $MgCl_2$) and 3 hr (no $MgCl_2$) determined by Chodosh et al. (12) using highly purified MLTF and substantially faster rates obtained by Sawadogo and Roeder (11) for a similar factor (USF) identified in a different chromatographic fraction. The slower rates of dissociation may be due to the use of a relatively crude fraction containing MLTF.

DNaseI footprinting of complexes formed in the absence or presence of HMGs 1 and 2

The stimulation of binding of MLTF by HMGs 1 and 2 was further investigated using DNaseI footprinting of MLTF-DNA complexes. The L-DNA fragment was 3' end-labelled at the AvaII site (i.e. coding strand); complexes were allowed to form in the absence of $MgCl_2$ and in the presence or absence of HMGs 1 and 2. DNaseI digestions were then done by addition of a mixture of $MgCl_2$ and DNaseI and incubation for 15 sec (in order to allow minimal time for DNA-transcription factor association in the presence of $MgCl_2$).

When incubations were done with increasing amounts of the MLTF fraction in the absence of HMGs 1 and 2, there was little protection of the MLTF binding region from DNaseI digestion (Figure 5). Minor increases in sensitivity to DNaseI, characteristic of MLTF binding, at sites flanking the binding site were seen (-69, -70) suggesting that only a small fraction of the DNA contained bound transcription factor. However, in the presence of HMGs 1 and 2 protection of the region from about bases -51 to -66 (as indicated by the box in Figure 5), characteristic binding of MLTF became

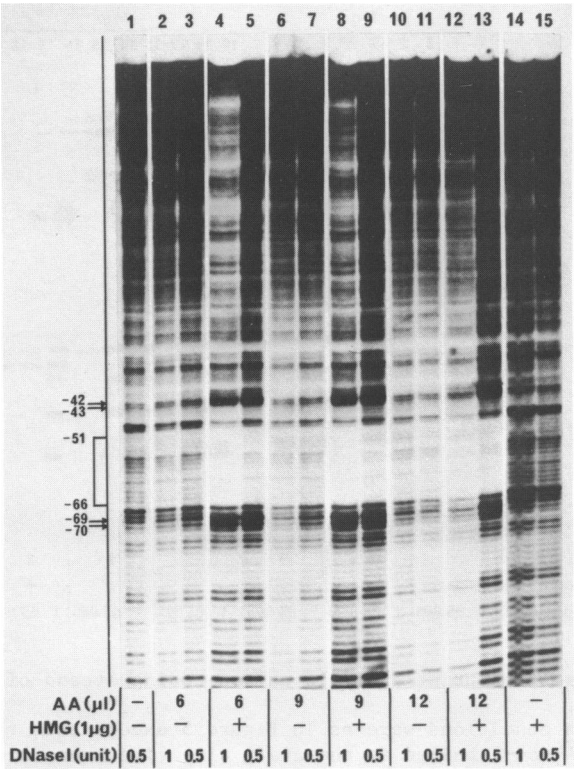


Figure 5. DNaseI footprinting analysis on the coding strand of MLTF binding to DNA.

Incubations for 40 min were done as before in the absence of $MgCl_2$ using 1.2 ng of 3' end-labelled L fragment, 400 ng poly(dI-dC), with or without HMGs 1 and 2 and with different levels of the AA fraction as indicated. DNaseI (1 or 0.5 units) and $MgCl_2$ to a final concentration of 5 mM were then added to the incubations and digestion allowed for 15 sec. The open box indicates the protected UPE region and arrows sites of enhanced sensitivity to DNaseI.

apparent; increased sensitivity to DNaseI of sites surrounding the protected region was also seen, particularly for the doublets of bands at -42, -43 and -69,70. In the presence of HMGs 1 and 2 alone there was no significant alteration in the pattern of sensitivity to DNaseI (tracks 14 and 15). The degree of protection and enhanced DNaseI sensitivity at particular sites are consistent with the stimulation of MLTF binding by HMGs 1 and 2 as seen in the gel binding assay.

DNaseI footprinting experiments were also done using DNA 5'

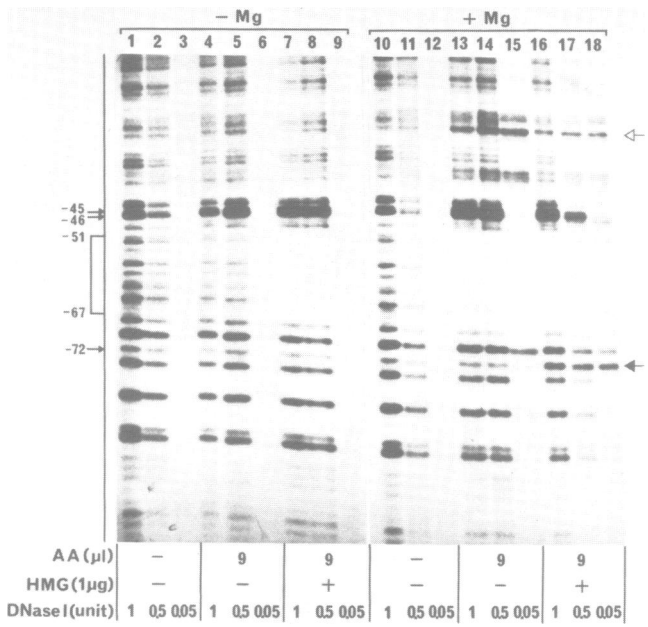


Figure 6. DNaseI footprint analysis on non-coding strand of MLTF binding to DNA.

Incubation conditions were as in Figure 5 except that the L fragment was 5' end-labelled at the AvaII site (i.e. non-coding strand) and incubations were set both in the absence (tracks 1 to 9) or presence (tracks 10 to 18) of 5 mM $MgCl_2$. The open box indicates the UPE protected region and arrows sites of altered DNaseI sensitivity. The open arrow on the right is at base -22 and the filled arrow at base -72.

end-labelled at the AvaII site (i.e. non-coding strand). Similar results were obtained in the absence of $MgCl_2$ (Figure 6), in that without HMGs 1 and 2 the MLTF binding site was not protected from DNaseI digestion and only a slight enhancement of the relative DNaseI sensitivity of sites (-45, -46) flanking the binding region was observed. In the presence of HMGs 1 and 2 clear protection of the binding region was again seen.

When DNA binding was done in the presence of $MgCl_2$, protection was evident both in the presence and absence of HMGs 1 and 2 (Figure 6). Additional sites of enhanced nuclease sensitivity were seen 3' to the protected region, around bases -34 and particularly base -22 (open arrow in Figure 6); control experiments have shown that cutting at these sites is due to magnesium-dependent nuclease activity present in the AA fraction. In the

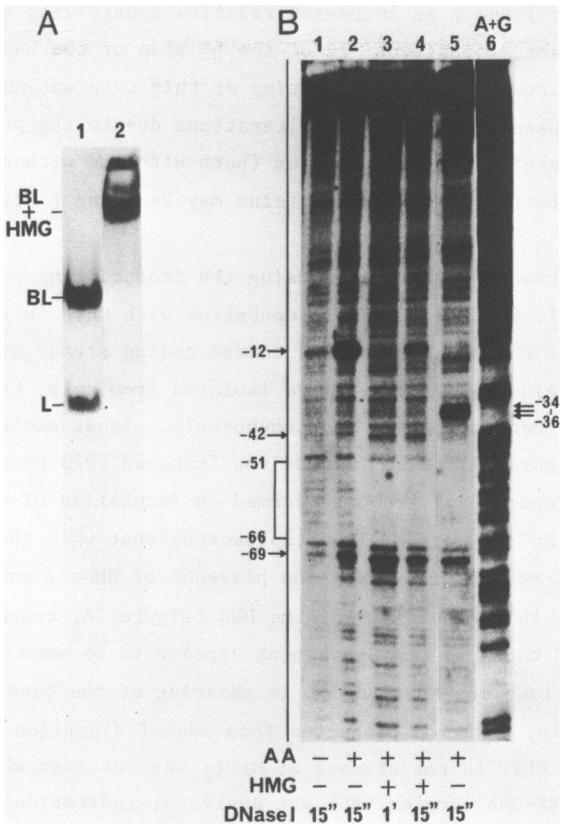


Figure 7. DNaseI footprints of complexes of DNA with MLTF and HMGs 1 and 2.

Incubations for MLTF binding to DNA were done for 40 min of the AA fraction, 1.2 ng of the L DNA fragment, 200 ng poly(dI-dC) with or without 1 μg of HMGs 1 and 2. After DNaseI treatment (0.05 units per reaction) incubation mixtures were separated on a preparative low ionic strength gel.

A. Tracks 1 and 2 show incubations done without and with HMGs 1 and 2 respectively (both incubations in the absence of $MgCl_2$, but migration of bands the same if $MgCl_2$ present). L and BL indicate the free and MLTF-bound L DNA fragment and BL + HMG the slowly-migrating band eluted from this track.

B. Denaturing gel electrophoresis of DNA eluted from preparative gel. Track 1, the unbound L fragment; track 2, the BL complex DNA; tracks 3 and 4, DNA from the BL + HMG complex; track 5, DNA from a BL complex isolated after incubation in the presence of 5 mM $MgCl_2$. The time of DNaseI treatment is indicated as are the UPE region (open box) and sites of enhanced DNaseI sensitivity.

presence of HMGs 1 and 2 an increased relative sensitivity to DNaseI digestion was seen at position -72 on the 5' side of the DNaseI-protected region (filled arrow, Figure 6); cutting at this site was not due to endogenous nuclease activity. The alterations due to the presence of HMGs 1 and 2 of the DNaseI footprint patterns (both with and without $MgCl_2$) suggested that both MLTF and HMG proteins may be bound to the same template DNA.

This was investigated further using the footprinting protocol described by Carthew et al. (8). Following incubation with MLTF (with or without HMGs 1 and 2) of DNA 3' end-labelled on the coding strand and DNaseI treatment, protein-DNA complexes were isolated from gels, the DNA extracted and analysed by denaturing gel electrophoresis. Bands excised for analysis are shown in Figure 7A; these include the "unbound" 528 bp L-fragment, the MLTF-bound L-fragment and the band formed on incubation of MLTF, the L-fragment and HMGs 1 and 2. It is noteworthy that when the L-fragment is used in the gel retardation assay the presence of HMGs 1 and 2 results in a large reduction in the mobility of the DNA (Figure 7A, track 2). The amount of HMGs 1 and 2 bound by the S-fragment appears to be much less, and only at high concentrations of HMGs 1 and 2 is smearing of the band up the gel seen.

Surprisingly, a region protected from DNaseI digestion in DNA associated with MLTF in the absence of $MgCl_2$ was not seen when the gel-purified MLTF-DNA complex (BL) was analysed; indications of binding were evident, however, from increased DNaseI sensitivity at positions -12 and -69 (Figure 7B, tracks 1 and 2). When the equivalent MLTF-DNA complex isolated after incubation in the presence of 5 mM $MgCl_2$ was analysed a clear protected region was observed (Figure 7B, track 5) with sites of increased DNaseI sensitivity at both 3' and 5' sites (-34 to -36 and -69). The complex from the MLTF-DNA-HMG incubation shows a clear footprint, similar to that seen when products of DNaseI digestion were analysed directly on a denaturing gel (Figure 5). However, the pattern of DNaseI sensitivity differs from that seen in the presence of $MgCl_2$, in that 3' sites partially protected from DNaseI (-49, -42) in the presence of $MgCl_2$ are not protected in the MLTF-DNA complex formed in the presence of HMGs 1 and 2.

DISCUSSION

Using the gel-retardation assay we have shown that the binding of the specific transcription factor MLTF to the upstream region of the adenovirus

major late promoter is stimulated by HMGs 1 and 2. Under both standard binding conditions (in the presence of $MgCl_2$) or sub-optimal conditions (in the absence of $MgCl_2$) a stimulation of 5 to 8-fold the rate of association of MLTF with promoter-containing DNA is seen (Figure 4). Stimulation of transcription from the adenovirus major late promoter by HMGs 1 and 2 previously seen using HeLa cell extracts in vitro (7) is likely therefore to be due in part to enhanced binding of MLTF. It is clear that this is not the only way in which HMGs 1 and 2 can stimulate transcription as increased transcription was seen from other promoters which do not contain MLTF binding sites - the VA1 and 2 RNA polymerase III genes and a chick feather keratin gene promoter (7). Recent results (Tremethick and Molloy, paper in preparation) also indicate that transcription from the adenovirus major late promoter is stimulated primarily in the initation phase, but to a lesser degree during elongation by HMGs 1 and 2. They may thus act to facilitate interaction of template DNA with a number of components of the transcription machinery. The levels of HMGs 1 and 2 used in these in vitro studies are high relative to those found in the nucleus. However, if HMGs 1 and 2 are confined to the active chromatin fraction at about 1 molecule per nucleosome the weight ratio of HMGs 1 and 2 to DNA would be 1:4. This compares with ratios of about 1:1 to 4:1 in the transcription and factor binding assays.

DNaseI footprint experiments have distinguished two different modes of binding of MLTF to promoter-containing DNA. In the presence of $MgCl_2$ binding is rapid and efficient and a clear region of protection from DNaseI is seen, bounded by sites of increased relative DNaseI sensitivity. This is consistent with the DNaseI footprint patterns seen by other workers (8,9,11,12). In the absence of $MgCl_2$ MLTF is still able to bind specifically to promoter-containing DNA to give a protein-DNA complex with the same electrophoretic mobility as that formed in the presence of $MgCl_2$, but the rate of association is substantially slower. The DNA in this complex is still accessible to DNaseI and no clear DNaseI-protected region is seen; this is true even when the DNA-MLTF complex is isolated from a gel following DNaseI treatment. Binding of MLTF in the absence of $MgCl_2$ is associated, however, with increased relative sensitivity to DNaseI at sites on the edge of the binding region. Increased sensitivity to DNaseI at position -69 is also seen upon MLTF binding in the presence of $MgCl_2$. While this association of MLTF with DNA in the absence of $MgCl_2$ is "loose"

in the sense that DNaseI protection is not observed it is quite stable as binding-chase experiments with excess unlabelled competitor DNA show little dissociation of MLTF over periods up to 15 hr (data not shown).

The presence of HMGs 1 and 2 alters the DNaseI digestion pattern seen when MLTF is bound to DNA either in the presence or absence of $MgCl_2$. In the absence of $MgCl_2$ a clear footprint is now observed in the presence of both MLTF and HMGs 1 and 2 though the protected region on the coding strand is not as large as when MLTF binds in the presence of $MgCl_2$ (Figure 7). HMGs 1 and 2 thus favour the formation of a protein-DNA complex in which accessibility of DNaseI is reduced to the sequence region shown to be important (by mutational studies, 13-17) for transcription from the adenovirus major late promoter. When MLTF binds in the presence of $MgCl_2$ this region is already protected but alteration to the pattern of DNaseI sensitivity caused by HMGs 1 and 2 is evident (Figure 6) at the 5' edge of the protected region.

The binding of HMGs 1 and 2 to DNA appears to be largely non-sequence specific except for a preference for A-T rich regions of low helix stability (23), and we have not observed alteration in the relative DNaseI sensitivity of any phosphodiester bonds in their presence. HMGs 1 and 2 alone also do not cause a discrete mobility shift in the gel retardation system only a smearing of the DNA band up the gel at high HMG concentrations. This effect is much greater for the longer DNA fragment and hence we have used the "S" fragment for most gel-retardation experiments. This suggests that binding of HMGs 1 and 2 may be cooperative and that when at low density on the DNA they dissociate readily under conditions of the gel-retardation assay.

Enhancement of the rate of MLTF binding may be a direct effect involving interactions of HMGs 1 and 2 with DNA and/or MLTF or an indirect effect through preferential interference with binding to DNA of other proteins in the AA fraction. Two indirect lines of evidence suggest that HMGs 1 and 2 and MLTF may be bound to the same probe DNA. HMGs 1 and 2 cause alterations to the footprint of MLTF - greatly increased protection from DNaseI in the absence of $MgCl_2$ and altered sensitivity to DNaseI on the 5' side of the protected region in the presence of $MgCl_2$. In addition, the slowly migrating band, BL + HMG (Figure 7A, track 2), contains bound MLTF, as evidenced by DNaseI protection, and is dependent on the addition of HMGs 1 and 2. HMGs 1 and 2 have been shown to have a domain structure (24,25) in which different regions are involved in DNA-binding and

in interactions with specific histones (26,27). It is possible therefore that the effects of HMGs 1 and 2 on the rate and configuration of MLTF binding and enhancement of transcription are mediated through either or both the DNA binding of HMGs 1 and 2 or through protein-protein interactions. Further experiments are needed to directly demonstrate whether HMGs 1 and 2 are associated with MLTF-DNA complexes and if so to investigate the nature of such an association.

Carthew et al. (8) have observed formation of a second complex (band II) specific for the upstream promoter element which migrates more slowly than the MLTF-DNA complex and which gives an identical footprint to the MLTF-DNA complex (band I). They suggest that additional protein(s) bind to the MLTF-DNA complex to form band II. We observe that formation of a slower-migrating band, which probably corresponds to their band II, is also stimulated by HMGs 1 and 2 (Figure 4). Indeed the presence of HMGs 1 and 2 causes an increase in the relative proportion of DNA present in the slower migrating band compared with the primary MLTF-DNA band. It will be of interest to study further the effects of HMGs 1 and 2 on the formation of this complex and the binding of other specific and general transcription factors such as Sp1 (29) and the TATA binding protein, TFIID (11).

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