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High mobility group chromosomal proteins bind to AT-rich tracts flanking plant genes

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Abstract

AT-rich sequences in the 5′ flanking regions of several plant genes have been shown to bind nuclear proteins, but the nature of these proteins has remained largely unknown. We report here that certain plant high mobility group (HMG) chromosomal proteins can interact specifically (in the presence of excess non-specific competitor) with AT-rich sequences located upstream of the pea ferredoxin 1 gene (*Fed-1*) and a member of the wheat *Em* gene family. Binding was observed with highly purified preparations of HMGa or HMGb, but not with HMGc or HMGd. HMG-DNA complexes were similar to one of the two types of *Fed-1* complexes we observed previously using pea nuclear extracts [7]. HMG binding to the *Fed-1* DNA was localized to a region containing AT-rich sequences; very similar sequences are present 5′ to *Em* and several other plants genes. Such sequences have been shown to bind unidentified nuclear proteins in a number of these systems. Binding experiments with a synthetic oligo (dA)•oligo (dT) probe and competition experiments with synthetic DNA polymers suggest that HMG binding may depend upon structural features of AT-rich DNA rather than being sequence-specific. We discuss the implications of these findings and suggest a role for HMG binding which is consistent with previous evidence linking HMGs with transcriptionally competent chromatin.

Previous studies have shown that dAdT tracts in mammalian genes often bind high mobility group (HMG) proteins *in vitro* [23, 26, 32], and similar data have recently been reported for a soybean gene [14]. HMGs are usually defined operationally as small (10–30 kDa) chromosomal proteins that are released from chromatin extracted with 0.35 M NaCl and are soluble in 2% trichloroacetic acid [17]. Furthermore, they are generally high in basic and acidic amino acids (ca. 25 mole% of each) and in proline (ca. 7 mole%). Although the term HMG applies principally to mammalian proteins, proteins meeting these operational criteria have also been characterized from both yeast and plant nuclei [21, 27]. Plant HMGs have been most thoroughly characterized from wheat [27] which possesses four proteins that meet the operational criteria for

HMGs. None of the wheat proteins exactly comigrate with vertebrate HMGs, but they do have similar mobilities through SDS-PAGE gels. Furthermore, both plant and animal HMGs are rich in both positively and negatively charged amino acids. Despite these similarities, however, peptide mapping, N-terminal sequencing and immunological studies show that plant proteins are structurally different from their putative animal counterparts [28].

Although the precise function of HMGs remains unclear, extensive studies have suggested a role for these proteins in the chromatin surrounding actively transcribed genes. This evidence has been extensively reviewed [6, 9, 28, 34] and can be summarized as follows. In chromatin separated into transcriptionally active and transcriptionally inert fractions based on differential sensitivity to micrococcal nuclease, HMGs are found in the transcriptionally active fraction. Extraction of HMGs from chromatin with 0.35 M NaCl abolishes the selective DNase I sensitivity of transcriptionally active genes. The sensitivity can be restored by reconstitution with purified HMGs. HMGs are preferentially released from chromatin by light DNase I digestion. Mononucleosomes can be separated into fractions enriched or depleted in transcribed sequences when passed through an affinity column consisting of HMGs bound to the column matrix. Somewhat longer oligonucleosomes can be similarly fractionated when passed through an affinity column consisting of HMG17 antibodies bound to the matrix. In gel shift assays, HMG14 and 17 have been shown to bind mononucleosomes containing transcribed sequences with a higher affinity than they bind mononucleosomes containing non-transcribed sequences.

Less is understood regarding the function of HMGs in the plant nucleus, but, despite the structural differences between the plant and animal proteins, preliminary evidence suggests that plant HMGs may also be associated with active chromatin [28]. Like their animal counterparts, wheat HMG proteins are released from chromatin that has been lightly digested with DNase I [31]. Furthermore, gel shift assays have shown that HMGa binds specifically to trimmed mononucleosomes [1].

We have recently found that extracts prepared from pea nuclei contain proteins which bind specifically to sequences in the region upstream of the pea *Fed-1* gene and which form two or more discrete DNA-protein complexes with different electrophoretic mobilities [7]. Preliminary deletion analysis showed that sequences necessary for the observed binding are located in a particularly AT-rich region between approximately 260 to 340 basepairs upstream of the transcription start site. Similar AT-rich sequences have been found upstream of a number of other plant genes [3, 12, 13, 15, 16, 18], including a member of the wheat *Em* gene family (R.S. Quatrano, personal communication). We report here that purified plant HMG proteins bind to AT tracts in the 5′ flanking region of the *Fed-1* and *Em* genes. Characteristics shared by the HMG-DNA complexes and one of the two major complexes we observed with crude pea nuclear extracts indicate that these complexes result from similar interactions.

Materials and methods

Proteins

HMG proteins were isolated from wheat embryo chromatin as described [27]. Wheat HMGs were fractionated by weak cation exchange HPLC using methods detailed [29]. HMGs from 7-day old pea seedlings were isolated from chromatin prepared by the method of Bonner *et al.* [2] as modified by Spiker *et al.* [31]. Pea nuclear extract was prepared from buds that were harvested in the light from etiolated seedlings that were germinated and grown in complete darkness for 7 days, as described by Elliott *et al.* [7].

Probe DNAs

The −492/−23 probe was a 472 basepair *Alu*I fragment extending from −492 to −23 relative to the *Fed-1* transcription start site [7]. This frag-

ment was cloned into the *Sma*I site of pBSM13- (Stratagene) to make pRE292. The −393/−256 probe was generated as follows. Insert from pRE292 was excised with *Eco*RI and *Hin*dIII and gel-purified [19]. The insert DNA was blunt-ended with Klenow DNA polymerase, digested with *Ssp*I and the resulting fragments shotgun-cloned into the *Eco*RV site of pKSM13+ (Stratagene). An *Ssp*I partial fragment extending from −393 to −256 was selected. The coding region probe has been previously described [7]. Labeled probes for gel shift assays were prepared by digesting with *Eco*RI and *Hin*dIII to excise the insert and then using Klenow DNA polymerase or avian myeloblastosis virus reverse transcriptase to fill in the recessed ends of both the insert and the vector [19]. Plasmid pBM113KP, which contained a 297 basepair fragment (−558 to −263) of a wheat *Em* has been previously described [20] and was prepared for gel shift assays by digesting with *Sal*I and *Kpn*I, end-labeled, and insert gel-purified. Plasmid pT_7A_{50} has been described previously [8] and was prepared for gel shift assays by digesting with *Bam*HI and *Eco*RI and end-labeled.

Gel shift assays

Gel shift assays were done as described by Elliott *et al.* [7]. 3 fmol of labeled probe DNA was included in each binding reaction. Samples were loaded onto gels, with the power on, at 30 s intervals. Competitor DNAs, poly(dIdC) • poly-(dIdC), poly(dAdT) • poly(dAdT), poly(dA) • poly(dT), poly(dA) and poly(dT) were obtained fom Sigma. 12.5 μg poly(dIdC) • poly(dIdC) was included in all binding reactions (25 μl) unless otherwise specified. All other competitors were used as described in the text.

Results

Unfractionated HMG proteins were prepared from wheat embryos as described [27], and assayed for specific binding to the *Fed-1* 5′ flanking sequences by gel mobility shift assays. Binding of the HMGs to the upstream region resulted in a complex that had a mobility through agarose gel that was significantly lower than the unbound DNA (Fig. 1a). However, as the amount of non-specific competitor (poly(dIdC) • poly(dIdC)) included in the binding reactions was increased, the mobility shift on the bound DNA was reduced, as the non-specific binding of HMGs to the labeled DNA was reduced. This trend continued until the amount of the competitor reached 1.6 μg, when two discrete retarded bands were observed. Further increases in the amount of poly(dIdC) • poly-(dIdC) caused no further reductions in mobilities of these complexes, indicating that these complexes were the result of specific binding of the HMGs to sequences contained in the *Fed-1* 5′ flanking region. This specific binding was even more dramatic when the amount of the HMGs was varied in the presence of a constant high level of non-specific competitor. As shown in Fig. 1b, increasing protein : DNA ratios resulted in a series of complexes showing discrete stepwise mobility shifts which presumably reflect specific additions of HMG proteins to the complexes. This pattern of discrete mobility shifts is quite unlike the pattern seen when HMGs bind non-specifically to bulk genomic DNA [1]. As demonstrated in Fig. 1c, no binding was observed with a probe from the coding region.

Since wheat HMG preparations are frequently contaminated with histone H1, it was important to determine if the protein : DNA complexes observed here were the result of H1 binding to the labeled probe DNA. Binding assays were done in which up to 4 μg of highly purified histone H1 was added to the reactions. No binding of wheat H1 to the promoter was observed under the conditions described here (Fig. 1d). To further characterize their binding activity, unfractionated HMGs were subjected to different treatments prior to the binding assays. Fig. 1e shows that the binding activity of wheat HMGs was completely abolished by digestion with proteinase K, whereas RNase A was ineffective. Pretreatment of the HMGs with heat (90 °C, 10 min) had no effect on binding activity.

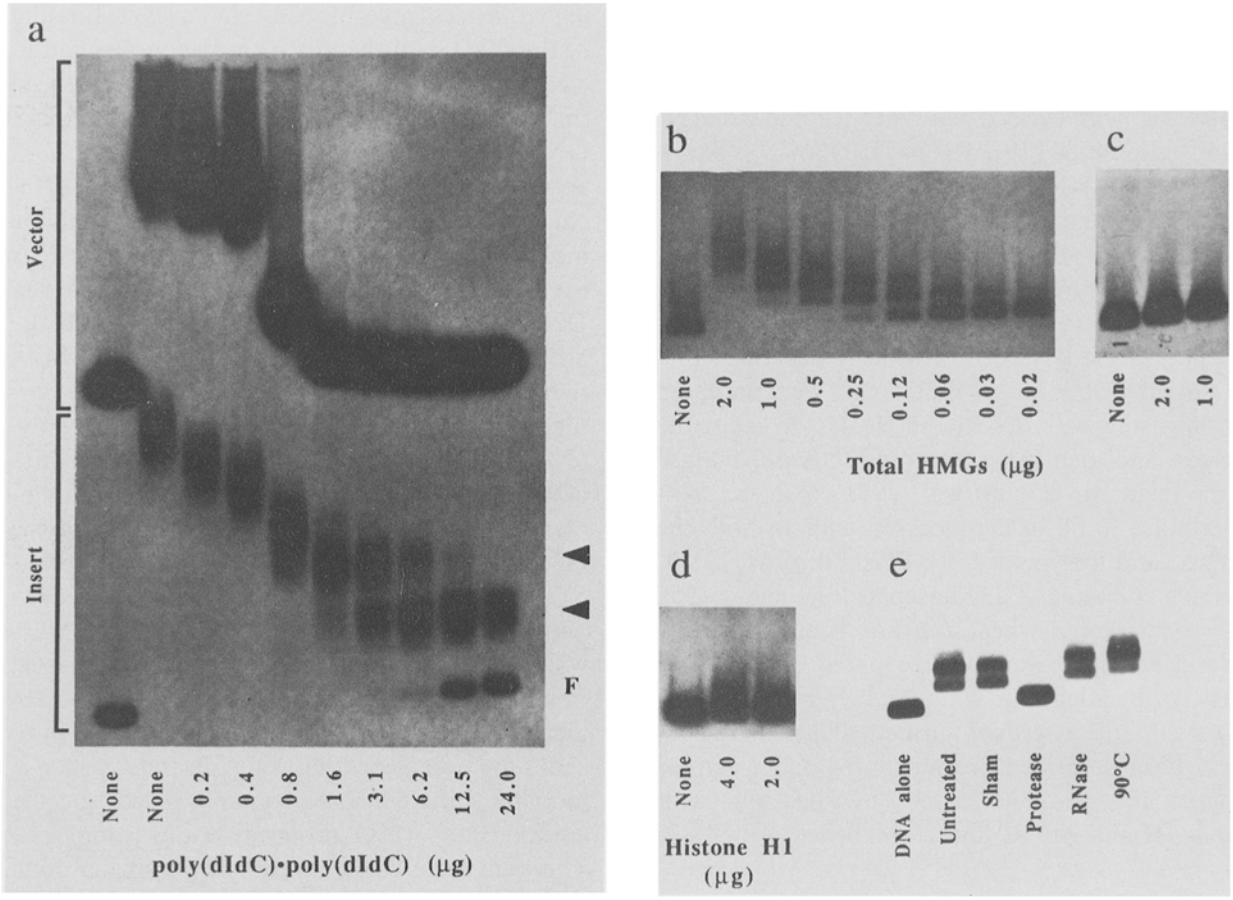

Fig. 1. Wheat HMGs bind specifically to sequences contained in the 5′ flanking region of *Fed-1*. Wheat HMG proteins were tested for binding to end-labeled probes containing *Fed-1* sequences in a gel retardation assay. The left hand lane in each panel contains a reaction incubated without added protein.

a. Wheat HMGs (0.5 μg) were assayed for binding to the −393/−256 probe in the presence of increasing amounts of poly(dIdC)•poly(dIdC). F, unbound probe; arrowheads, specific HMG : DNA complexes. Poly(dIdC)•poly(dIdC) used in b–e was 12.5 μg.

b. Effects of differing amounts of wheat HMGs on binding to the −492/−23 probe.

c. Binding of HMGs to a probe from the coding region (+231 to +528) of *Fed-1*.

d. Binding of wheat histone H1 to the −492/−23 probe.

e. Effects of various pretreatments on the binding activity of wheat HMGs. HMGs (0.5 μg) were incubated on ice (Untreated), incubated at 37 °C without additions (Sham), incubated at 37 °C with proteinase K or RNase A (1 mg/ml), or incubated at 90 °C for 10 min before being assayed for binding activity.

To determine which of the four characterized wheat HMGs were capable of specific interactions with the *Fed-1* upstream region, pure HMGa and HMGb and a mixture of HMGc and HMGd were prepared [29] and used in binding reactions with both the −492/−23 and the coding region probes. Both HMGa and b are capable of forming discrete complexes with *Fed-1* sequences (Fig. 2). Although the major band that resulted from the reactions incubated with HMGc and d appeared to have a retarded mobility this altered mobility in fact was a result of the power being on during loading of the gel. Thus, only a single, minor retarded complex was observed when HMGc and d were assayed for binding to this probe. This minor complex can

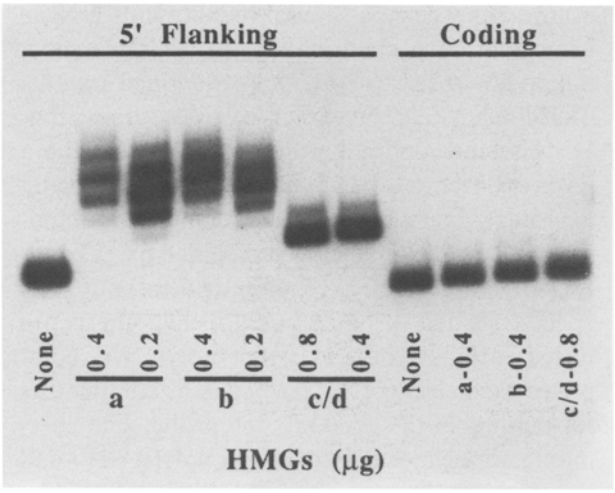

Fig. 2. Binding of purified wheat HMGs to sequences in the *Fed-1* 5′ flanking region. HMGs were fractionated as described [29], and assayed for binding to the −492/−23 and coding region probes.

probably be attributed to contamination of the HMGc/d preparation by either HMGa or b. None of the HMGs were able to bind to the coding region probe.

Figure 3a shows that the unfractionated wheat HMGs bind to the shorter −393/−256 probe and form a series of complexes with discrete mobilities, similar to those seen with the −492/−23 probe. Thus, wheat HMGs bind to the same region shown by deletion analysis to be required for the binding of pea nuclear proteins [7]. Nuclear proteins which meet the operational criteria for HMGs, and which also crossreact with polyclonal antibodies raised against wheat HMGs [30] (data not shown), were isolated from pea seedlings and tested for binding activity. Figures 3b and 3c show that proteins in both the wheat and pea HMG preparations interact

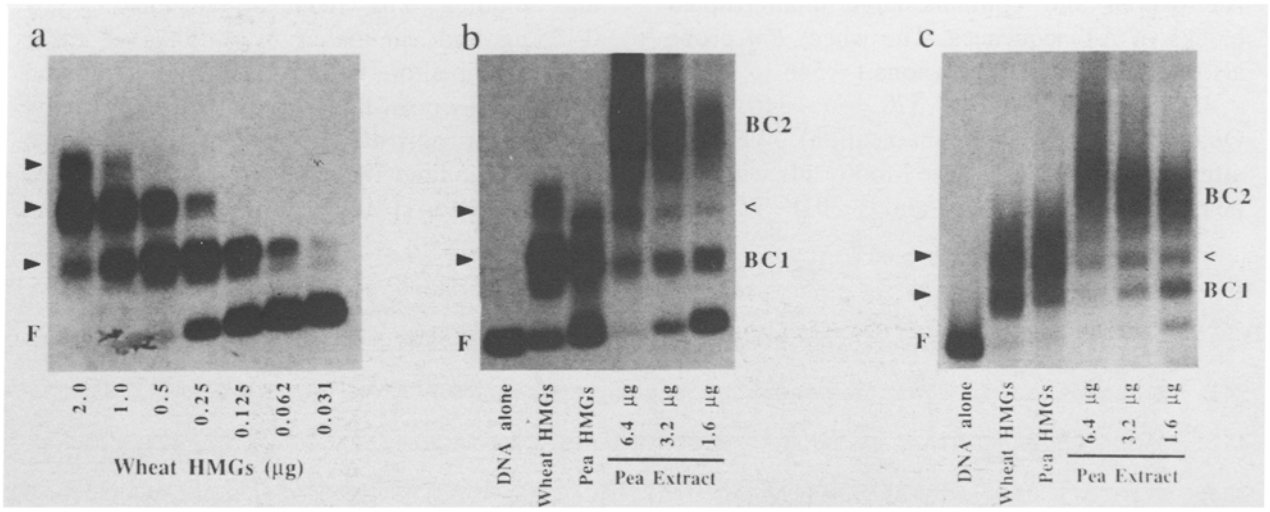

Fig. 3. Nuclear proteins from both pea and wheat interact with sequences 5′ to either the pea *Fed-1* or a wheat *Em* gene.
a. Binding of wheat HMGs to the *Fed-1* −393/−256 probe. Wheat HMGs were included in binding reactions containing the end-labeled −393/−256 probe, as indicated. The positions of the unbound probe (F) and specific HMG-DNA complexes (arrowheads) are shown.
b. Comparison of binding by wheat HMGs, pea HMGs and pea nuclear proteins to the *Fed-1* −393/−256 probe. Wheat HMGs (0.5 μg), pea HMGs (1.0 μg) or differing amounts of a pea nuclear extract were added to binding reactions prior to gel shift assays with the −393/−256 probe. The positions of the unbound probes (F), specific HMG-DNA complexes (filled arrows) and the BC1-like complexes (<) are indicated, as well as the BC1 and BC2 complexes observed with pea extracts.
c. Comparison of binding by wheat HMGs, pea HMGs, and pea nuclear proteins to sequences 5′ to a wheat *Em* gene. Experiment is the same as in *b* except that the labeled probe is a 297 basepair *Kpn* I-*Sal*I fragment (−559 to −263) found upstream of a wheat *Em* gene (R.S. Quatrano, personal communication).

with both the −393/−256 probe from the pea *Fed-1* gene and with a region 5′ to *Em*, a wheat abscisic acid-regulated gene [20]. In both cases, the resulting complexes comigrate with BC1, one of the two major types of complexes observed with crude pea nuclear extracts [7]. Even though additional HMG-DNA complexes can be observed at very high levels of HMGs (Fig. 3a), none of these complexes have the same mobility as BC2, the second major complex formed with pea nuclear extracts. The only HMG-DNA complexes with mobilities similar to that of BC2 are non-specific complexes, such as those shown in Fig. 1a. Since all of the binding reactions shown in Fig. 3 contain far more poly(dIdC) than is needed to suppress such non-specific interactions, formation of BC2 probably depends on factors other than HMGs.

Analysis of the sequences upstream of the *Fed-1* transcription start site has shown the region encompassed by the −393/−256 probe to be remarkably AT-rich (Fig. 4). This region is 83% AT overall and contains large uninterrupted blocks of AT sequences. The wheat *Em* probe also has three AT-rich regions (−546 to −505 −485 to −413, and −378 to −346; R.S. Quatrano, personal communication). We have attempted to obtain DNase I footprints with two pea probes (−393/−256 and −301/−256). In both cases we see a general reduction in DNase I sensitivity, especially in the most AT-rich region, but do not observe short, sharply defined regions. These results can be explained by assuming that HMGs bind randomly to different sites within a given AT-rich region, so that no part of the region is protected on all molecules. Such random binding might reflect a general preference for AT-rich DNA rather than a requirement for any one specific sequence. To address this hypothesis, we tested various synthetic competitor DNAs composed exclusively of AT basepairs as competitors for binding of the −393/−256 probe. The alternating copolymer poly(dAdT) • poly(dAdT) and the homopolymer poly(dA) • poly(dT) were chosen because they represent the two extremes of AT sequence specificity. These competitors were added to the reactions in addition to the 12.5 μg of poly(dIdC) • poly(dIdC) included in all binding assays. As shown in Fig. 5a, both poly(dAdT) • poly(dAdT) and poly(dA) • poly(dT) were effective competitors for HMG binding. Binding was reduced considerably by 0.125 μg and eliminated by 1.25 μg of either dAdT competitor. Up to 1.25 μg of additional poly(dIdC) • poly(dIdC) or poly(dGdC) • poly(dGdC) or poly(dG) • poly(dC) had no such effect on binding. This ability of dAdT sequences to compete for HMG binding depends on some

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                                                    gagctctttgtgacatatgtcaaagagggagttcaacgtagctgttctata
-620  actcttgatgatgtaatctgattaagataaagataccttaagcccccataagtcagataattcaaaaatatctagttaaa
-541  gtgtcaatcaatcattgaatcaagcagatcatcttgaagtttgaaagCTCACATAGTCATGTGCTAAATATTTTAGAGT
-462  GACCATCTTAAATTGTAAAAGGTAAAGAGTATGTCTTGAAGTACTAAGACAACCACACGCACTTAAAATATTTTCAAAC
-383  CTTCTCTTCCTTTATTAAAACTTATTAAAAACCTATCCAGACATTTATTTATGTAATTGATTAAAAAGTATTTCTAATC
-304  AATATTTTAATTAAGTTAATTTAATAAAAATGTAATATCTTATATCAATATTATTGTATTTTTTTATCTATAAAAAAAC
-225  CTTAAACACAGAATGAGTATTGTATATGAAGACTTGAGTAATTAGTTACCACAGTGCACAATACTTCATTTTTAAATAC
-146  TATAAGGTGAAGATGGTGCCAACAAGGATTCACCAACAATATGTGGTGATAAAAGAGATAGATAACCTGAGGCATAAAA
 -67  CAATGCCACCTGGCAGATAGGGTTGCATGCAGTTCATATAGCAGCttgttccaaccacactatttcc              +1
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Fig. 4. The 5′ flanking region of the *Fed-1* gene is AT-rich. Uninterrupted AT blocks of 6 or more base pairs (required for the binding of mammalian HMG-I; [26]) are shaded. Sequences in the −494/−23 probe are shown as upper case letters, sequences outside this are lower case. The smaller probes (−393/−256 and −301/−256) are underlined: thin line, sequence found only in the −393/−256 probe; heavy line, sequence common to both. Nucleotide position is relative to the transcription start site [7].

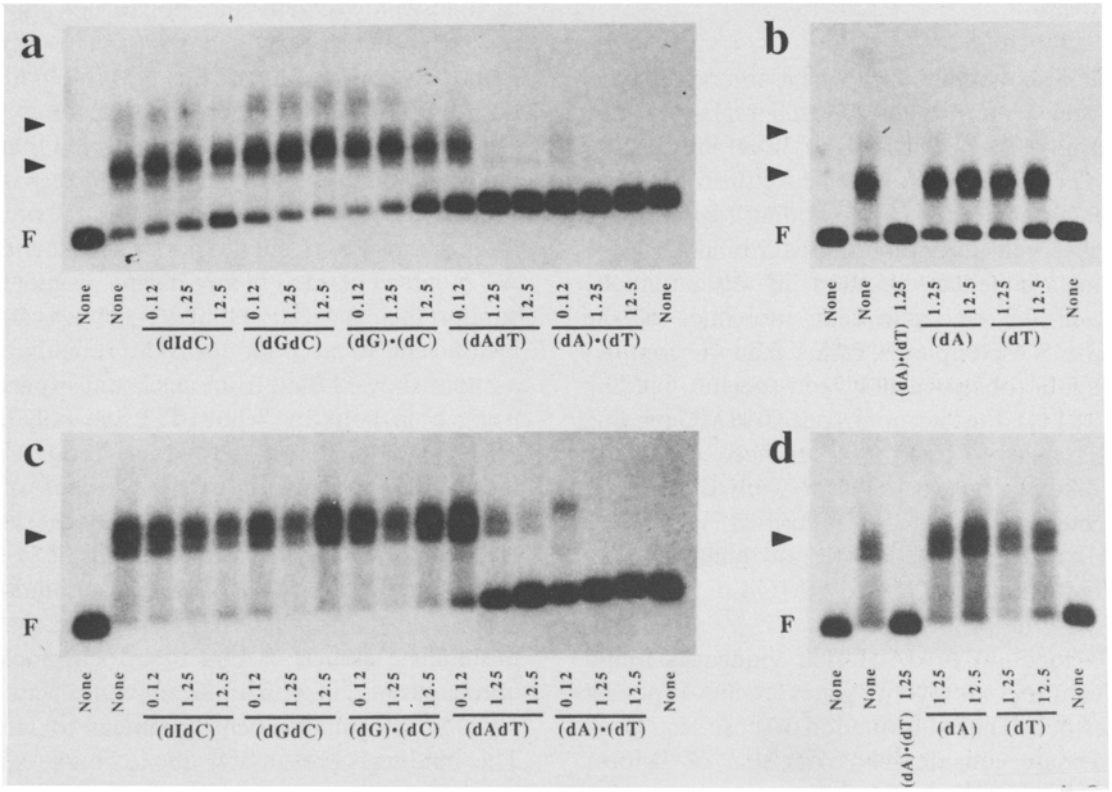

Fig. 5. Effects of additional synthetic DNA competitors on the binding of wheat HMGs to *Fed-1* sequences and to a synthetic AT-rich sequence. In all panels (a,b,c,d), the outermost lanes contain no protein.

a. Indicated amounts (μg) of each synthetic DNA were included in binding reactions in addition to the normal 12.5 μg poly(dIdC) • poly(dIdC) prior to gel shift assays with the − 393/ − 256 probe.

b. The ability of AT-rich sequences to compete for HMG-binding to the − 393/ − 256 probe is double-strand-specific. Indicated amounts of each competitor were included in binding reactions in addition to the normal 12.5 μg of poly(dIdC) • poly(dIdC) prior to gel shift assays.

c. Same as for *a* except that the labeled probe DNA is the AT probe from pT_7A_{50}.

d. Same as for *b* except that the labeled probe DNA is the AT probe from pT_7A_{50}.

feature found only in double-stranded DNA, since neither poly(dA) nor poly(dT) were effective competitors even when present at concentrations 10-fold higher than those at which the double-stranded competitors completely eliminated binding (Fig. 5b).

We further tested the binding of wheat HMGs to a synthetic AT-rich probe prepared by excising a fragment composed almost exclusively of AT sequence from plasmid pT_7A_{50} [8]. This plasmid was originally constructed as one of a set of plasmids used to investigate the effects of poly(A) tails of differing lengths on the fate of synthetic RNAs introduced into protoplasts. The probe used here was the 'poly(A) tail' region of pT_7A_{50} and was made up of two 25-mers of oligo(dA) • oligo(dT) separated by a single GC basepair. As shown in Fig. 5c and 5d, purified HMGs formed specific complexes with the AT probe. Since the AT probe has essentially the same sequence as the synthetic poly(dA) • poly(dT) that competed for HMG binding to the − 393/ − 256 *Fed-1* probe, it is likely that HMG binding to both probes is similar. As was the case with the − 393/ − 256 probe, only poly(dAdT) • poly(dAdT) and poly(dA) • poly(dT) were able to to compete for HMG binding to the AT probe.

Discussion

We have shown that both wheat and pea HMGs bind specifically to an AT-rich region located upstream of the pea *Fed-1* gene. In gel shift assays, binding of HMGs was specific for the 5′ flanking sequences. No shift of the coding region probe was observed. In addition, HMG binding to the flanking sequences resulted in discontinuous shifts in the electrophoretic mobilities of the HMG-DNA complexes, rather than the continuous shifts observed for non-specific binding (Fig. 1a [1]). Furthermore, specific HMG binding was sensitive to the presence of either poly(dAdT) • poly(dAdT) or poly(dA) • poly(dT), but not to poly(dIdC). Purified HMGa and HMGb were both capable of binding independently, but HMGc and HMGd did not bind to any of the probes used.

The function of dAdT-rich sequences found upstream of eukaryotic genes remains poorly understood. The conformation of these sequences can deviate considerably from that of B-form DNA [5, 11, 22] and it has been shown that the presence of dAdT tracts in regions flanking genes enhances the levels of expression of these genes in yeast [4, 24, 33]. It has been suggested that structures peculiar to dAdT tracts, rather than a strict sequence specificity *per se*, are required for interaction of AT motifs with certain mammalian HMG proteins. For example, Soloman *et al.* [26] showed that HMG-I, then known as α-protein, will bind to any run of six or more dAdT basepairs. In addition, Jacobsen *et al.* [14] showed that HMG-like proteins from soybean will bind to different AT-rich sequences found upstream of a nodulin gene. A similar preference for structure over sequence has been reported by Bustos *et al.* [3] who showed that changing part of an AT motif found upstream of the bean phaseolin gene from ATATAAT to TATATTA had no effect on the binding of an unidentified protein present in bean nuclear extracts, although replacing the natural sequence with CGCGCCG eliminated binding. The importance of structure over a strict sequence specificity for HMG binding is also supported by the observation that both poly(dAdT) • poly(dAdT) and poly(dA) • poly(dT), but not the single-stranded poly(dA) or poly(dT) homopolymers, compete for HMG binding to both the *Fed-1* and AT probes (Fig. 5).

Recently, Grasser *et al.* [10] reported that partially purified HMG proteins from maize, partially protected both the CCAAT and TATA boxes in footprinting assays but did not protect the several AT motifs contained in a zein gene promoter. To support their suggestion that AT-richness alone is insufficient to account for HMG binding, the authors showed data from a gel shift experiment using both poly(dA) • poly(dT) and poly(dG) • poly(dC) competitors. However, these experiments were performed in the absence of any non-specific competitor DNA and the results therefore probably reflect non-specific HMG-DNA interactions. All HMGs can bind non-specifically to bulk DNA [1] presumably by a mechanism similar to that of cytochrome c, an unrelated protein which has an amino acid composition and charge density similar to HMGs. This binding is presumably due to charge : charge interactions between cytochrome c and the negatively charged DNA. In our experiments, we included an excess of poly(dIdC) to suppress such non-specific interactions [25], the effects of which can be seen in Fig. 1. The gel shift competition results of Grasser *et al.* are very similar to the reduction of non-specific binding caused by increasing concentrations of poly(dIdC) that we observed in Fig. 1a. We believe the effect of poly(dG) • poly(dC) in their experiments is comparable to the effects of poly(dIdC) in ours, and thus that their data do not show specific binding to GC-rich sequences. In contrast, binding to AT-rich sequences is not suppressed by high levels of non-specific competitor. We have no evidence of HMG-binding to any *Fed-1* sequences outside of the AT-rich sequences found well upstream of the CAAT and TATA boxes and Grasser *et al.* did not address the issue of whether or not the footprints they observe can be attributed to HMGs as opposed to other components in their partially purified preparations.

HMGs have been found in all eukaryotes so far examined [21] and have been shown to interact

with dAdT motifs in mammalian genomes [26, 32]. In plants, AT-rich sequences very similar or identical to those we have identified have been reported in the upstream regions of a variety of genes from a number of species. Such sequences have often been shown to interact with nuclear proteins and regions containing them have been implicated in controlling gene expression [3, 18, 20]. The nuclear extracts used in these binding studies were all prepared with $\geq$ 350 mM salt and can be presumed to contain HMG proteins.

Pea HMG preparations interact with the same sequences as do the wheat HMGs. In addition, the pea HMG-DNA complex comigrates with BC1, one of the two major complexes obtained with pea nuclear extracts (Fig. 3b), indicating that formation of this complex may involve HMGs. The hypothesis that BC1 formation in pea nuclear extracts involves HMGs is further supported by the relative insensitivity of this binding activity to thermal denaturation. Incubation of pea nuclear extracts or purified wheat HMGs at 90 °C for 10 minutes prior to their addition to binding reactions fails to reduce the formation of either BC1 or the HMG-DNA complexes. In addition, competition experiments suggested that BC1 formation involved a relatively abundant protein with a relatively low affinity for *Fed-1* sequences [7]; both of these characteristics are consistent with HMG interactions with dAdT-rich sequences in mammalian genomes [26, 32].

The identity of the protein(s) which form BC2, the second complex observed with pea nuclear extracts, remains unclear. Although we cannot yet exclude the possibility that BC2 results from an interaction of multiple HMG-DNA complexes with each other, both the susceptibility of this complex to self-competition and the thermal instability of the protein(s) involved in its formation suggest that BC2 involves one or more proteins other than HMGs. However, since we have never observed BC2 without BC1 there is a possibility that formation of a BC1-type HMG-DNA complex must precede BC2 formation. This scheme is especially attractive since it suggests a biological role for HMGs consistent with the experimental evidence associating them with transcriptionally competent chromatin [28]. In this scenario, HMGs would act as molecular 'bookmarks' by binding to AT tracts near enhancer-like sequences. The abundance of HMGs in the nucleus would favor their binding to most such sites. Additional factors might then interact with these complexes, or might displace the HMGs to produce more specific complexes. In either case, the initial binding of HMG proteins can be visualized as helping to maintain a chromatin structure accessible to transcription factors.

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