A novel activity of HMG domains: promotion of the triple-stranded complex formation between DNA containing (GGA/TCC)$_{11}$ and d(GGA)$_{11}$ oligonucleotides

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ABSTRACT

The high mobility group protein (HMG)-box is a DNA-binding domain found in many proteins that bind preferentially to DNA of irregular structures in a sequence-independent manner and can bend the DNA. We show here that GST-fusion proteins of HMG domains from HMG1 and HMG2 promote a triple-stranded complex formation between DNA containing the (GGA/TCC)$_{11}$ repeat and oligonucleotides of d(GGA)$_{11}$ probably due to G:G base pairing. The activity is to reduce association time and requirements of Mg$^{2+}$ and oligonucleotide concentrations. The HMG box of SRY, the protein determining male-sex differentiation, also has the activity, suggesting that it is not restricted to the HMG-box domains derived from HMG1/2 but is common to those from other members of the HMG-box family of proteins. Interestingly, the box-AB and box-B of HMG1 bend DNA containing the repeat, but SRY fails to bend in a circularization assay. The difference suggests that the two activities of association-promotion and DNA bending are distinct. These results suggest that the HMG-box domain has a novel activity of promoting the association between GGA repeats which might be involved in higher-order architecture of chromatin.

INTRODUCTION

The high mobility group proteins HMG1 and HMG2 are abundant, chromatin-associated proteins whose function is unknown. The two proteins belong to one class of the HMG family of proteins that is a collection of small acidic proteins extracted from chromatin with 0.35 M NaCl (1). Extensive studies of HMG1/2 proteins revealed that they bind preferentially to DNA of irregular structures in a sequence-independent manner (2–4). This binding occurs through several domains called HMG boxes, which were first identified by the comparison of HMG1 with UBF, a protein involved in regulating the expression of genes encoding ribosomal RNA (2,5). Two homologous segments of an 80-amino acid sequence, box-A and box-B, are present in HMG1/2 (6,7). Interestingly, similar domains are found in many other proteins such as LEF-1, TCF-1, SRY and the related SOX proteins (4,8–12). They comprise single HMG-box domains and have the additional property of sequence-specific binding to linear DNA. The HMG-box proteins have a capacity to unwind DNA structure by bending (13,14). Such distortions are probably important to facilitate the action of transcription factors bound nearby (4,15,16). For this reason they are considered as architectural proteins in nuclei (4,17).

Homopurine/homopyrimidine sequences are abundant in eukaryotic genomes and some of them consist of tandem repeats such as (GGA/TCC)$_{n}$ (18–20). The sequences are known to take a non-B DNA conformation which may provide some signals for a variety of DNA transactions (21–23). We previously reported that d(GGA)$_{11}$ oligonucleotides, but not d(GAA)$_{11}$, dimerize in vitro probably through guanine–guanine base pairing (24). The oligonucleotides also bind to double-stranded DNA containing (GGA/TCC)$_{11}$, resulting in a triple-stranded complex (25). The complex is presumed to form a unique structure, consisting of a homoduplex between the two GGA repeats and a single-stranded loop of the TCC repeat. Such a D-loop-like structure is distinct from the triplex model (26) applicable to the complex formed between the (GGA/TCC)$_{11}$ repeats and d(GGT)$_{11}$ oligonucleotides, because the two complexes exhibit distinct association kinetics and DNase I footprints (25).

Search for binding protein by Southwestern analysis revealed that HMG1 preferentially binds to the homoduplex between d(GGA)$_{11}$ oligonucleotides. This suggested a possible involvement of this architectural protein in the triple-stranded complex formation, although the binding may have resulted from the binding preference of HMG1 to irregular DNA structures. The

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finding prompted us to investigate effect of HMG1 or HMG-box proteins on the complex formation. We found that GST-fusion proteins containing HMG-boxes of HMG1, HMG2 and SRY enhance the association of triple-stranded complexes between DNA containing the (GGA/TCC)11 repeat and (GGA)11 oligonucleotides. The present paper describes details of this enhancement. The results obtained here provide evidence for a novel activity of the HMG domain: promotion of complexes between GST-repeats. Biological relevance is also discussed.

**MATERIALS AND METHODS**

**Synthesis of GST-fusion proteins**

Recombinant clones were constructed using pGEX2T (27). They are capable of directing the synthesis in *Escherichia coli* of box-AB, box-A and box-B of HMG1 and HMG2, and the HMG box of SRY. Sets of primers carrying BamHI and EcoRI tags were designed according to each sequence (28–30) to produce GST-fused proteins of those areas. Primer sequences are as follows: 5′-AGGATCCAAAGGATCCATCTAAAGAAC-3′ and 5′-TTGAAATTCCTTTCGCTGATCGAG-3′ for HMG1 box-AB; 5′-AGGATCCAAAGGATCCATCTAAAGAAC-3′ and 5′-TTGAAATTCCTTTCGCTGACATCAGG-3′ for HMG1 box-B; 5′-AAGGATCCATCGGCTCCCAAGCGG-3′ and 5′-TTGAAATTCCTTTCGCTGACATCAGG-3′ for HMG2 box-AB; 5′-AAGGATCCATCGGCTCCCAAGCGG-3′ and 5′-TTGAAATTCCTTTCGCTGACATCAGG-3′ for HMG2 box-A and 5′-TTGAAATTCCTTTCGCTGACATCAGG-3′ for HMG2 box-B.

**Association assay**

Double-stranded DNA of 162 bp was synthesized in the presence of [γ-32P]dCTP with PCR using the pUC118 carrying d(GGA/TCC)11 between the XhoI and BamHI sites. Primer sequences used are 5′-TTTCTCCAGTCACGAC-3′ (M4) and 5′-CAGGAAACAGCTATGCAC-3′ (RV). The labeled fragments having (GGA/TCC)11 (1 nM) was incubated in the absence or presence of HMG-domain proteins (6–750 nM) with d(GGA)11 (50 or 100 nM) at 37°C for 30 min in 10 μl of a buffer containing 50 mM NaCl, 10 mM Mg2+, 0.2 mM DT, 20 mM HEPES (pH 7.9) and 6% glycerol, followed by cooling to 4°C in the course of 30 min by a programmable thermal cycler (ASTEC Inc., Japan) (25). The products were subjected to electrophoresis in 5% nondenaturing polyacrylamide gels (PAGE) with and without the digestion with proteinase K at a concentration of 50 μg/ml for 2 h at 4°C. The electrophoresis was performed in 1× TBE (50 mM Tris–borate, pH 8.3, 1 mM EDTA) buffer containing 50 mM NaCl and 10 mM Mg2+ at 4°C. T4 gene 32 protein and cytochrome C were purchased from Boehringer Mannheim Co., Germany.

**DNase I protection assay**

The triple-stranded complexes were obtained as described above except for the use of 10 nM of the 162 bp fragment containing (GGA/TCC)11. The DNA bound with HMG1 was obtained by incubation with box-AB (10 nM DNA and 750 nM protein: a protein–DNA molar ratio of 75:1) in a buffer containing 20 mM HEPES (pH 7.9), 50 mM NaCl, 2 mM MgCl2, 0.2 mM DT for 15 min at 20°C. The complexes and the 162 bp fragment were subjected to limited DNase I digestion in 250 μl of a buffer containing 10 mM HEPES (pH 8.0), 40 mM KCl, 5 mM MgCl2, 0.5 mM DT, 2 μg of sonicated calf thymus DNA and 2.5 U of DNase I (Takara, Japan). The digestion was allowed for 5 min at 20°C and terminated by addition of 50 μl of a buffer containing 10 mM Tris–HCl (pH 7.4), 20 mM EDTA, 100 μg/ml RNA and 0.1% SDS. The DNA was recovered with ethanol precipitation after phenol treatment. Levels of the digestion were analyzed by primer extension method using Circum Vent thermal cycle dyeoxy DNA sequencing system (New England BioLabs) except 0.2 μM each of dNTPs used. The M4 and RV primers were 5′-GTTTTCCAGTCACGAC-3′ and 5′-CAGGAAACAGCTATGCAC-3′. Amplified DNA was cleaved with *SalI* and subsequently subjected to analysis as described previously.

**Circularization assay**

Circularization assay was carried out as described (13). 32P-labeled DNA fragment with EcoRI sticky ends was synthesized as follows: PCR was carried out in the presence of [γ-32P]dCTP using the pUC118 carrying (GGA/TCC)11 as a template and a set of primers: 5′-GTGTTTCCAGTCACGAC-3′ and 5′-CAGGAAACAGCTATGCAC-3′. Amplified DNA was cleaved with HindIII and then ligated into pUC118 to give pUC118/C. Circular DNA was purified by CsCl/ethidium bromide gradient centrifugation and digested with *SalI* and *XhoI*. The circular DNA was then ligated with the linearized pGEX2T in the presence of T4 Polynucleotide kinase and T4 DNA ligase. After transformation into *E. coli* DH5α, the transformed cells were grown to an A600 of 0.4 and then lysed using lysozyme/MgCl2. The lysates were subjected to electrophoresis on a 6% polyacrylamide–8 M urea gel in parallel with sequencing ladder. The bands were visualized by autoradiography.
with EcoRI and fragments of 120 bp were purified after polyacrylamide gel electrophoresis. The fragment (1 nM) was incubated without and with GST-fusion proteins containing HMG-box in 10 µl of a buffer containing 50 mM HEPES–NaOH (pH 7.5), 50 mM potassium glutamate, 10 mM Mg-acetate and 1 mM ATP for 10 min at 30°C. T4 DNA ligase (0.6 Weiss U, Takara, Japan) was then added and incubated for 10 min followed by inactivation of the ligase by shifting to 65°C for 15 min. Reactions were subsequently incubated with 1 U of exonuclease III (Takara, Japan) for 45 min at 37°C to remove linear ligation products. The reaction products were extracted with phenol and ethanol precipitated. The nuclease-protected products were recovered by centrifugation and subjected to electrophoresis on a 5% polyacrylamide gel in Tris–borate–EDTA (pH 8.3) buffer which was then dried and autoradiographed.

**Gel mobility-shift assay**

Gel-mobility shift assays were carried out as described (15,32). DNA containing the SRY recognition sequence, AACAAAG, used here was produced as follows. Two complementary sequences, 5′-GATCCGGGGAGACTGAGAACAAAGCGCTCTT-3′ and 5′-CTAGAAGAGCGCTTTGTTCTCAGTCTCCCG-3′, were synthesized and annealed, and then the fragment was cloned into BamHI and XhoI of pUC118. PCR was carried out in the presence of [α-32P]dCTP with M4 and RV primers using the plasmid DNA as template.

**RESULTS**

**Effect of HMG1/2 on triple-stranded complex**

Plasmids capable of directing the synthesis in *E.coli* of box-AB, box-A and box-B of HMG1 were constructed by cloning the relevant sequences of the mouse cDNA in pGEX2T. They encoded for amino acids K2-K172, K2-K89 and N92-K172, respectively. Each GST-fusion protein was purified to near homogeneity and used for association assay. Also, three GST-fusion proteins of HMG2 were synthesized in a similar manner. Figure 1A shows the effect of HMG1 on the formation of a triple-stranded complex. 32P-labeled double-stranded DNA (1 nM) containing the (GGA/TCC)11 repeat was synthesized with PCR and incubated with a low concentration (50 nM) of d(GGA)11 oligonucleotides. Products in the mixture were then separated by polyacrylamide gel electrophoresis on a 5% polyacrylamide gel in Tris–borate–EDTA (pH 8.3) buffer which was then dried and autoradiographed.

![Figure 1](image1.png)
a property of giving a shifted band even without the proteinase K treatment. The box-A of HMG2 and the box-B of HMG1 seemed to have that property, though levels were much weaker than that of the HMG2 box-B. These differences may reflect differences in their binding affinity to the DNA. Addition of anti-HMG1 antibodies in the reaction mixture inhibited the association promotion (Fig. 1 C), indicating that HMG1, but not contaminated components in the protein preparation, enhanced the association.

It is known that single-stranded DNA binding proteins such as the T4 gene 32 protein facilitate the annealing of complementary DNA strands by exposing single-stranded regions and are involved in recombination and replication (34). Since HMG1/2 can bind to single-stranded DNA, the property may be responsible for stimulation of the association. Therefore, effects of two DNA-binding proteins, the gene 32 and cytochrome C, on the association were examined. Either of them did not affect the complex formation in a range of similar concentrations (Fig. 1 D). This suggests that the binding property of HMG1/2 to single-stranded DNA itself may be irrelevant or insufficient for the stimulation activity.

Properties of HMG1 domains

Properties of HMG1 for the association were investigated in this assay using the box-AB protein. Figure 2A shows an analysis of time course in the presence of 10 mM Mg2+. Fifty percent complex formation required 40 min without the protein but was achieved within only 10 min in the presence of box-AB (750 nM). The formation of triple-stranded complex was dependent on the Mg2+ concentration; 2 mM Mg2+ did not give the complex. At least 6 mM was required for 50% binding under this condition. However, the addition of box-AB reduced the Mg2+ requirement to 2 mM (Fig. 2B). Therefore, the assay done under the 2 mM Mg2+ gave more striking difference. Figure 2C shows effect of d(GGA)11 oligonucleotide concentrations. The protein was able to reduce the minimal concentration required for the band shift; at the concentration of as low as 1.6 nM of d(GGA)11, duplex containing (GGA/TCC)11 was little converted into the triple-stranded DNA (lane 2). Upon addition of the box-AB protein, however, the complex was formed under the same condition (lane 8). These results demonstrated that the enhancing activity of the box AB protein for the triple-stranded complex formation is to reduce association time and requirements of the Mg2+ and oligonucleotide concentrations.

Figure 3A shows effect of protein concentrations. The box-AB protein promoted the association to give a shifted band at a concentration as low as 6 nM; the protein–DNA molar ratio was 6:1 (lane 3), whereas at that concentration both box-A and box-B failed to enhance (lanes 7 and 11, respectively). The box-B did not show the shifted band even at the concentration of 30 nM (lane 12). This suggests that the order of the promoting activity is box-AB, box-A and box-B. Figure 3B shows that native HMG1 purified from Ehrlich ascites tumor cells had the enhancing activity, although its activity was weaker than that of GST-fusion proteins. The three kinds of HMG2-fusion proteins also exhibited a similar order of activity (data not shown).

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DNase I footprinting

Structural analysis was carried out using DNase I. The complexes between DNA containing (GGA/TCC)11 and d(GGA)11 formed in the presence and absence of HMG1 were partially digested with DNase I, and the sensitivity was measured by the extension method using 32P-labeled primers as described in Materials and Methods. Figure 4 shows gel electrophoretic patterns of the PCR products. The complex formed in the absence of HMG1 exhibited increased sensitivity for both strands of the duplex and a hypersensitive site in the vicinity of the repeat region of the GGA strand (lanes 2 and 6), consistent with our previous result (25). The same pattern was observed for the complex formed in the presence of HMG1 (lanes 4 and 8). This sensitivity suggests that the triple-stranded complex is not the triplex previously defined (26) but a D-loop-like structure (25). Effect of the HMG1 binding was also examined; the box-AB did not show significant protection of the repeat and flanking sequences (lanes 3 and 7), but slightly increased sensitivity in the repeat region of the GGA strand (lane 3).

DNA bending activity

HMG-boxes are known to have the capacity to modulate DNA structure by bending (13, 14). Since promotion of the association may reflect the bending activity, their relationship was examined.
DNA bending was measured by the T4 DNA ligase-catalyzed circularization of the short DNA fragment having the repeat in the middle and the EcoRI site at both ends. Figure 5 shows the ligation products in this assay. In the presence of T4 DNA ligase alone, the fragment formed a few broad bands (lane 2). Those bands are not circular molecules induced by the binding because they are cut into shorter fragments by the digestion with exonuclease III (lane 3). Upon addition of the box-AB of HMG1, however, the ligation provided a band of closed-circular molecules resistant to the exonuclease digestion (lane 4). DNA fragments in the band were isolated and their sizes were determined by polyacrylamide gel electrophoresis after the digestion with HindIII and SmaI in the vicinity of EcoRI ends. The fragment containing the EcoRI site was observed (data not shown), indicating that major HMG1-induced ligation product is a monomer circular. At the box-AB concentration of 30 nM (a protein–DNA molar ratio of 30:1), the circle was detected but levels decreased rapidly at the concentrations >150 nM (lanes 5 and 6). The box-B of HMG1 gave a maximal level of the product at the concentration of 750 nM (lane 9). At 200 nM it showed a super-shift pattern (lane 10). Similar shift patterns were observed for DNA fragments containing the SRY-recognition sequence of AACAAAG (9) (lanes 4 and 5). These results indicated that the fusion protein of SRY binds to both sequences.

Figure 6B shows an association assay of the SRY. The protein was also able to facilitate the complex formation (lanes 9 and 10). The concentration required was similar to that of the box-B of HMG1 (lanes 5 and 6). Signals on start and shifted bands were weaker at protein concentrations >100 nM. Mixing the two proteins at the concentration of 200 nM each did not reduce band signals (data not shown). The reason for reduced signals is hence unclear. The obtained results suggest that the HMG-box domain possess the association-promoting activity, irrespective of sources that the box is derived from.

It was also tested whether SRY has a DNA-bending activity for DNA containing the (GGA/TCC)$_{11}$ repeat (Fig. 2C). The HMG box of SRY binds to linear DNA containing the target sequence and produces a

**Activity of HMG-box derived from SRY**

The SRY protein plays a primary role in male-sex determination and contains a single HMG-box (30,37). In contrast with HMG1/2, it is a regulatory protein with a limited cell-type distribution and with sequence specificity for the binding. Therefore, effect of its HMG-box was studied. GST-fusion protein containing the HMG-box of SRY was synthesized, and its binding was first examined in a gel mobility-shift assay (Fig. 6A). The SRY bound to DNA fragments containing the (GGA/TCC)$_{11}$ and provided a discrete band at the concentration of 40 nM (lane 9). At 200 nM it showed a super-shift pattern (lane 10). Similar shift patterns were observed for DNA fragments containing the SRY-recognition sequence of AACAAAG (9) (lanes 4 and 5). These results indicated that the fusion protein of SRY binds to both sequences.
Figure 5. Circularization of DNA containing the (GGA/TCC)$_{11}$ repeat promoted by the box-AB, box-A and box-B of HMG1. The position of closed circular DNA is marked by an arrowhead. Lanes 1–3: no protein added; lanes 4–6, box-AB of HMG1 added at indicated concentrations; lanes 7–9: box-B of HMG1; lanes 10–12, box-A of HMG1. Samples in lanes 2–12 were treated with T4 DNA ligase and those in lanes 3–12 digested with exonuclease III. This assay was carried out as described in Materials and Methods.

sharp bend (9). However, such bending was not detected by the DNA ligase-catalyzed circularization assay (13,35,38). As shown in Figure 6C, the circularization assay revealed that GST-fusion protein of SRY did not give circular molecules (lanes 2 and 3). Addition of the protein to the HMG1 box-B reaction mixture did not inhibit the circularization (lanes 4–7), demonstrating that the SRY preparation used did not contain inhibitor or DNase activity. The result suggests that there is some difference between the two domains in bending activity for DNA containing the repeats, consistent with previous reports (13,35).

**DISCUSSION**

The present studies with HMG1 and HMG2 indicate that these abundant nuclear proteins are capable of promoting a triple-stranded complex formation between DNA containing the (GGA/TCC)$_{11}$ repeat and oligonucleotides of d(GGA)$_{11}$ (Fig. 1). Association of the complex is probably due to G–G base pairing between the two GGA repeats, because d(GGA)$_{11}$ oligonucleotides form homoduplex in a parallel orientation through that pairing (24). The promoting activity of HMG1 is to reduce association time and requirements of Mg$^{2+}$ and oligonucleotide concentrations, which is demonstrated by several experiments (Fig. 2): (i) analysis of reaction time was done under the condition containing 10 mM Mg$^{2+}$ where the complex was spontaneously formed. The result reveals that the binding shortens time in the incubation needed for the complex formation even under that condition; (ii) the Mg$^{2+}$ concentration required is decreased by the binding. The association occurs at a Mg$^{2+}$ concentration of 2 mM, at which concentration no complex is observed without the box-AB protein; and (iii) the required oligonucleotide concentration is also reduced. The complex is clearly seen at 1.6 nM of d(GGA)$_{11}$, ~2-fold excess of the double-stranded DNA, only upon addition of the box-AB protein. Such enhancement is not observed in other two single-stranded DNA-binding proteins that can promote the annealing of complementary DNA strands (Fig. 1D). These results substantiate the ability of the HMG proteins to facilitate the complex formation.

Our studies also reveal that the activity of promoting the complex is maintained by one of the two HMG-box domains.
present in HMG1 and HMG2. Each of the HMG-box domains consist of ~80 amino acids (6,11,12). It seems likely, however, that the box-AB protein of HMG1 has an activity stronger than the box-A and the box-B (Fig. 3A). This may be attributed to the presence of two domains as one unit. On the other hand, native HMG1 exhibits a weaker activity (Fig. 3B) which may be due to the presence of the acidic domain in the C-terminal end (39). Actually, a fusion protein of HMG1 box-BC (C indicates an acidic domain) displayed an activity weaker than that of box-B (data not shown).

HMG box is present in many other proteins which comprise the HMG-box family of proteins (4,11,12). There are two subfamilies. Members with multiple HMG domains including HMG1/2 and UBF present in all cell types. They show low sequence specificity for the binding. The others have single HMG domains and have a restricted cell-type distribution. They recognize specific sequences. SRY, a protein to determine the male differentiation (30,37), is one of the proteins belonging to the latter group. GST-fusion protein carrying the SRY HMG-box domain can bind to DNA containing (GGA/TCC)\textsubscript{11} and facilitates the association between the DNA and d(GGA)\textsubscript{11} oligonucleotides as efficiently as the box-B of HMG1 does (Fig. 6). This suggests that the association-enhancing activity is not restricted to the HMG-box domains derived from abundant nuclear proteins of HMG1/2 but is common to HMG domains from other members of the HMG-box family of proteins involved in transcription and cell differentiation as well.

The HMG-box is known to have the capacity to modulate DNA structure by bending (13,14). The bending may be an activity responsible for the association promotion. Therefore, their relationship was examined by measuring the T4-DNA ligase-catalyzed circularization of a short DNA fragment having the repeat (Fig. 5). The box-AB and box-B of HMG1 exhibit circularization activities much higher than the box-A does, consistent with previous reports (13,35). On the other hand, the box-A shows an association activity similar to or rather higher than that of box-B. This discrepancy may be due to different detection sensitivity of the two assays but suggests that the association promotion does not simply reflect the bending activity. A similar difference is also observed in the case of HMG box of SRY. The protein fails to bend DNA in our circularization assay, consistent with a previous report (35), whereas it facilitates the association between the DNA containing (GGA/TCC)\textsubscript{11} and d(GGA)\textsubscript{11} as efficiently as the box-B of HMG1 does (Fig. 6). These results suggest that the association-promoting activity and the bending activity are distinct and that the HMG-box domain has a novel property of promoting the association between GGA repeats in addition to its abilities to bind to irregular DNA and to bend DNA.

DNase I protection analysis has indicated that the binding of HMG1 to linear DNA does not affect the DNase I cleavage patterns for most DNA because of the low sequence specificity except for DNA of irregular structures (17,40,41). The DNA containing (GGA/TCC)\textsubscript{11} bound with the box-AB did not give the protection pattern, either, but showed hypersensitivity in the repeat region of the GGA strand (Fig. 4A). The hypersensitivity might reflect the HMG1-induced distortion or unwinding of DNA. If it is the case, the unwinding would provide separation of the GGA- and TCC-strands which facilitates the association of two GGA-repeats by increasing their accessibility.

The finding that HMG1/2 promote the association between the (GGA/TCC) repeats and single-stranded DNA of homologous sequence suggests that the association involves two double-stranded DNA harboring such repeats. This implication is possible, because the repeats have a property of adopting two single-stranded loops, as revealed by nuclease S1 digestion experiments (18,20). We are currently testing this possibility in vitro and have obtained a positive result in preliminary experiments (Mishima et al. unpublished). The promotion activity may be regarded as one analogous to the function of certain protein chaperons, which stabilize a polypeptide in a conformation that is appropriate for subsequent assembly, but would be unstable without a chaperone (42). This may provide another clue to the function of HMG1 and HMG2. There are other tandem repeats that can form four-stranded DNA complexes: telomeric DNA (43–45), guanine-rich sequences (46) and (CA/TG)n repeats (47). HMG1 preferentially binds to the latter repeats (17,47). These four-stranded complexes could take place with aid of HMG1/2 among many chromosomal regions because such repeats are abundant in the nucleus (49). It is possible that those complexes act as architectural elements constituting chromosomal domains or providing condensation (or decondensation) of the 30 nm fiber. The abundance of HMG1 and HMG2 in a nucleus is consistent with a general requirement for those conformations, particularly during times of chromatin remodeling in cells undergoing DNA replication and mitosis.

Sequence-specific association between the GGA repeats might be implicated in the initiation of the pairing of homologues during meiosis and a pairing mechanism of recombination or repair. The former was already suggested for G-rich four-stranded DNA (46). The latter speculation is consistent with the presence of a unique triple-stranded structure in the recombination process that is similar to the complex formed between (GGA/TCC)\textsubscript{11} and d(GGA)\textsubscript{11} (48,49). The finding that microsatellites comprising (GGA/TCC)n repeats are recombinogenic as deduced by their polymorphisms (50) may also support this speculation.

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