Pairing of DNA Fragments Containing (GGA:TCC)\textsubscript{n} Repeats and Promotion by High Mobility Group Protein 1 and Histone H1* 

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DNA stretches comprising homopurine:homopyrimidine constitute approximately 1% of mammalian genomes, and some of them are highly conserved. Those sequences in chromatin as well as naked DNA are sensitive to single strand-specific nucleases and possess a unique property of taking a non-B DNA conformation (1–4). The property may serve candidate motifs for adaptation of special structures such as triple-stranded complexes in chromosomes, which have been implicated in transcriptional regulation, recombination, and stabilization of chromosomes. We have investigated tandem repeats of (GGA:TCC)\textsubscript{n} that belong to this type of homopurine:homopyrimidine DNA. Sequences of such repeats are abundant in mammalian genomes, each up to 50 bp long, and the tracts exist at approximately 10\textsuperscript{4} copies per genome (5). It was found that d(GGA)\textsubscript{11} oligonucleotides, but not d(GAA)\textsubscript{11}, form a parallel-stranded homoduplex in vitro probably through guanine:guanine base pairing (6). A triple-stranded DNA complex is also formed between d(GGA)\textsubscript{11} oligonucleotides and double-stranded DNA containing (GGA:TCC)\textsubscript{11} (7).

High mobility group proteins, HMG1 and HMG2, are abundant non-histone chromosomal proteins. There is approximately one molecule of HMG1 per 5 and 20 nucleosomes in rat tissues and in rabbit thymus and chicken cells, respectively (8–10). They have two homologous segments, A and B, termed the HMG box (11, 12). The HMG box domain interacts with the minor groove of the DNA helix (13) and has a property of binding to irregular DNA structures in a sequence-independent manner (13–15) and the capacity to modulate DNA structure by bending (16–18). HMG1/2 are incorporated in chromatin and may have a structural role in organizing chromatin. Several functions have been proposed, the stimulation and inhibition of transcription (19–22) and a role in nucleosome assembly and disassembly (23, 24). However, their functions still remain open to question. Other chromosomal proteins, histones H1 and H5, share several properties with HMG1/2, which include requirement of linker DNA for stable incorporation into chromatin (25, 26) and selective interactions with the core histones (27). The linker histone also plays a key role in directing the formation of higher order structure in a nucleosomal array.

We have recently reported a novel activity of HMG1 that HMG domains are able to enhance the formation of triple-stranded DNA complex between DNA containing (GGA:TCC)\textsubscript{11} repeat and (GGA)\textsubscript{11} oligonucleotides (28). Since double-stranded DNA containing (GGA:TCC)\textsubscript{11} repeat can be unwound, forming two single-stranded DNA regions, it is possible that the double-stranded DNAs associate with each other and constitute DNA-DNA pairing complexes in the presence of HMG1 protein. The present paper investigates this issue. Here, we demonstrate the formation of such DNA-DNA complexes and describe involvement of HMG1 and histone H1 in the pairing.

EXPERIMENTAL PROCEDURES

Preparation of \textsuperscript{32}P-Labeled and Non-labeled Double-stranded DNA Fragments—Six DNA fragments used for association were synthesized with PCR using three template DNAs in the absence and presence of [\textsuperscript{32}P]dCTP. The template for four fragments, L, M1, M2, and S, shown in Fig. 4A was pUC118 DNA carrying the d(GGA:TCC)\textsubscript{11} repeat between the XbaI and BamHI sites. Four primers were used: F, 5'-GCTTTCCCACTCACGACAG-3'; F118, 5'-ATTCGAGCTCTCGGTACCCGG-3'; R, 5'-CAGAAACACGCTATGAC-3'; and R118, 5'-GCATGCCGTCGCGTCACT-3'. L fragment was synthesized with F and R primers, M1 with F118 and R, M2 with F and R118, and S with F118 and R118. The template for G5 fragment has been isolated by screening Sau3A partially digested mouse genomic DNA library, and the sequence is shown in Fig. 1A. F-G5 (5'-AGAACCACTGCTCTGGCGCAGT-3') and R-G5 (5'-CCTAATACGACCTCACACCCAC-3') were used to amplify the 172-bp DNA fragment. TCE (transcriptional control element) DNA fragment of 294 bp was directly amplified from mouse genomic DNA with PCR using primers, F-TCR (5'-GATTTCTACGATCCGAAA-3') and R-TCR (5'-TGGTCGCGATCTCAGGAA-3') (29).

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Proteins—GST fusion proteins containing box AB, A, and B of HMG1 were prepared as described previously (28). GST fusion histone H1 containing the whole 211 amino acid residues was prepared by constructing a recombinant clone as described (28), in which the insert DNA fragment was obtained from mouse genomic DNA using two primer sequences, F-H1 (5'-AAGGATCCGGGCTGCTCTGCTG-3') and R-H1 (5'-TTGAAATCTCTATTTTTTCTGGTGCTG-3'). Histones H1, H3, H4, cytochrome c, and proteinase K were purchased from Boehringer Mannheim (Germany). Escherichia coli single strand binding protein was obtained from Sigma.

Association Assays—The 32P-labeled double-stranded DNA (ds-DNA) fragments (1 nM) were incubated in 10 μl of buffer containing 50 mM NaCl, 0.2 mM dithiothreitol, 20 mM HEPES, pH 7.9, and 6% glycerol at 37 °C for 1 h, with or without non-labeled ds-DNA at various concentrations, and in the presence or absence of indicated proteins (7, 28). When we examined the effects of distamycin (Sigma) and actinomycin D (Sigma) on the DNA-DNA complex formation, 32P-labeled DNA and these antibiotics were preincubated at 37 °C for 10 min in 9 ml of a buffer and then added 1 μl of protein (500 nM) or non-labeled ds-DNA (100 nM) to the reaction mixtures, followed by incubation at 37 °C for 1 h. After incubation, the reaction mixtures were digested with proteinase K at a concentration of 75 μg/ml at 4 °C for 2 h. The heat stability of the DNA complexes was examined by heat-treating aliquots at indicated temperatures (°C) for 5 min. The products were separated on 5 or 4% native polyacrylamide gel electrophoresis (PAGE) in 1 × TBE (50 mM Tris borate, pH 8.3, 1 mM EDTA) buffer containing 50 mM NaCl and 10 mM MgCl2 at 4 °C under circulation of the buffer. The gels were dried and autoradiographed.

Preparation of Synthetic Size Markers—The five single-stranded DNAs, a to e, shown in Fig. 2A were prepared with five plasmid DNAs containing TG repeat, TG repeat, Sry sequences, and pUC118 sequence itself. The primers were as follows: 5'-GGGAGACTG(AACAAAG)2CGCTCT-3' (Sry sequence) was inserted into BamHI and XhoI sites of pBR322 and into BamHI and XhoI sites of pUC118. (CT)10,CCGGCCCTC(CT)10 (sequence itself) was cloned into BamHI and XhoI sites of pUC118. (TCC)11, sequence was into the same sites of pUC118 as described above. Using these plasmid DNAs, ds-DNAs having a non-labeled phosphate at either of the 5'-end are synthesized with PCR using phosphorylated and non-phosphorylated primers. The primers are as follows: 5'-OH-F-pBR and 5' R-PH-pBR for (α) pBR-Sry; 5'-P-F-pBR and 5'-OH-R-pBR for (β) pBR-TG, 5'-P-F and 5'-OH-R for (γ) pUC-Sry, 5'-OH-F and 5'-P-R for (η) pUC-TG, 5'-OH-F and 5'-P-R for (ε) pUC. Here, sequences of primer for pBR are 5'-AAGAATTCCATCTTGCCGCGGCGG-3', and for pUC are 5'-AAAGAATTCCATCTTGCCGCGGCGG-3'. Primers F and B are the same ones used for the ds-L fragment described above. Synthesized ds-DNAs were then digested with 12 units of λ exonuclease (Life Technologies, Inc.) at 37 °C for 30 min in 0.5 ml of buffer containing 67 mM glycine KOH, pH 9.4, 2.5 mM MgCl2, 25 μg of bovine serum albumin. Resultant size-markers were purified with a Centricon 30 (Amicon), and then the 5'-end was phosphorylated with [γ-32P]ATP and polynucleotide kinase. 32P-Labeled single-stranded α and β fragments were heated to 90 °C for 3 min in 10 μl of 10 mM Tris, pH 7.4, 1 mM EDTA (TE) containing 0.1 mM NaCl and cooled on ice, followed by 5% PAGE. Heteroduplex (α/β) was purified from the gel and annealed to γ fragment to yield heterotramer (α/β/γ) as described above. Gel-purified heterotrimer was then annealed to δ and ε fragments to produce the heterotetramers α/β/γ/δ and α/β/γ/ε, respectively, which were purified with 5% PAGE.

Results

Formation of DNA-DNA Complexes between DNAs Containing (GGA:TCC)11 Repeats in the Presence of GST-HMG1 Protein—Four kinds of DNA fragments containing (GGA:TCC)11 repeat were synthesized with PCR and used to examine DNA/DNA association between them (Fig. 1A). Fragment L of 101 bp has a (GGA:TCC) repeats in the middle, flanked by unique sequences at both sides, and M2 and M1 have shorter unique sequences at the right and left sites, respectively. Fragment S has shorter sequences at both sides. Association was carried out by incubation of 32P-labeled L fragment (1 nM) with excess amount (100 nM) of non-labeled M1, M2, or S fragments in the presence of GST-HMG1 protein. The products were analyzed by 5% polyacrylamide gel electrophoresis (Fig. 1B). Without non-labeled double-stranded DNA (ds-DNA), the 32P-labeled ds-DNA (indicated as 32P-L on the right side of the panel) did not provide any prominent extra bands in the gel. On the other hand, addition of non-labeled ds-DNA fragments in the incubation mixture gave slowly migrating bands. The bands showed the different migrations depending on the sizes of non-labeled DNA fragments added (indicated as L/M1, L/M2, and L/S on the right side of the panel). Since electrophoresis was performed after proteinase K treatment of the DNA-protein complexes, the slowly migrating bands represented DNA-DNA complexes without HMG1 protein.

Fig. 1C shows a gel-shift band of DNA-DNA complex between the 32P-labeled L and non-labeled S fragments formed in the presence of GST-HMG1 segments containing box AB, box A, or box B. The results indicated that proteins consisting of single HMG boxes were able to promote the DNA-DNA complex formation between DNAs containing (GGA:TCC)11 repeats. Without the protein or in the presence of GST-tag itself (see Fig. 6B), the extra band was not detected.

Comparison of Gel Mobility in DNA-DNA Complexes—DNA markers taking different structures, heterodimer, heterotrimer, and heterotetramer, were constructed, and their mobility in a gel was compared with that of the DNA-DNA complex between DNA containing (GGA:TCC)11 repeat. Five single-stranded DNA fragments shown in Fig. 2A were synthesized by PCR amplification followed by digestion with λ exonuclease. They consist of repeat sequences in the middle and pBR or pUC sequence at the right and left sites, respectively. Fragment L possesses complementary sequences to pUC-TCC(A) and pUC(Y) possesses complementary sequences to pUC-TCC(β) and pUC(ε). The 32P-labeled heterodimer (α/β) formed between α and β was gel-purified and annealed with γ fragment to yield heterotrimer α/β/γ where the two internal repeat sequences of α and γ were complementary. Then, the δ and ε fragments were incubated with the heterotrimer to form heterotetramer-1 α/β/γ/δ and

![Fig. 1. DNA-DNA complex formation promoted by GST fusion HMG1 proteins.](http://www.jbc.org/)

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heterodimer-2 α/β, heterotrimer α/β/γ, and heterotetramer-2 α/β/γ/ε, respectively. These markers, together with the L-M2 complex, were analyzed for the gel mobility shifts by running on 5% PAGE (Fig. 2B). The DNA-DNA complex (L/M2 in lane 7) showed a similar position to those of the heterotetramers (lanes 4 and 5). This suggests that the complex consists of two ds-DNA and the pairing takes place probably at the GGA repeat sequences.

**Conditions for the DNA/DNA Pairing with GST Fusion HMG1 Protein**—Effect of the HMG1 concentration on the DNA/DNA pairing was examined (Fig. 3A). The shifted band is detected at concentrations of more than 188 nM GST-HMG1 AB in the presence of 100 nM non-labeled ds-DNA. Fig. 3B displays the effect of the concentration of carrier non-labeled ds-DNA. At least 12.5 nM non-labeled ds-DNA was required for the complex formation in the presence of 750 nM GST-HMG1AB. These results show a dependence of the complex formation on the concentrations of GST-HMG1AB protein and ds-DNA concentration.

**Heat-stability of the DNA-DNA Complex**—GST-HMG1AB (100 nM) was treated with 75 mg/ml proteinase K at 4 °C for 2 h, followed by heat treatment for 5 min at the temperature (°C) indicated below each lane, and then run on 5% PAGE. Arrowhead indicates an unexpected product induced by heat treatment at 70 °C and also observed by heat treatment at 80 and 90 °C (data not shown). Structure of this complex is not clear. – indicates no addition of protein or ds-DNA.
Fig. 3, C and D, shows the time course for the complex formation and the heat stability of the complex formed, respectively. The DNA/DNA molecules paired between 32P-L and non-labeled M2 were observed at 5 min incubation and increased gradually up to 60 min of incubation (Fig. 3 C). The DNA-DNA complex was retained at the heat treatment of 60 °C, although the band intensity of the complex decreased at more than 50 °C (Fig. 3 D).

Pairing of Genomic DNAs Containing (GGA:TCC)_n Repeats—To examine whether or not genomic DNAs containing (GGA:TCC)_n repeats form DNA pairing, we isolated a DNA fragment containing (GGA:TCC)_n repeats, named G5, by screening a genomic DNA library. Another fragment was obtained by PCR amplification of DNA sequence that had been isolated as transcriptional control element (TCE) (29). Their sequences are shown in Fig. 4, A and B. Both contain more than 11 repeats of GGA:TCC, but the repeat units are imperfect and degenerate. 32P-Labeled ds-DNAs and non-labeled DNAs were prepared with PCR and subjected to the assay for DNA pairing.

As shown in Fig. 4, C and D, these DNAs were able to provide DNA-DNA complexes under the same condition used for the DNA containing (GGA:TCC)_{11} repeat. However, there was a difference, i.e. a band of the complex was detected even in the absence of GST-HMG1AB protein when non-labeled ds-DNA was present at 100 nM.

Sequence Dependence for DNA Pairing—The three DNA fragments containing (GGA:TCC)_n repeats, L (M1), G5, and TCE, have different arrangements of repeats and different flanking sequences. L and M1 have one (GGA:TCC)_{11} repeat array flanked by unique pUC sequences, and G5 has many arrays of short GGA:TCC repeats and degenerate repeats between them. TCE possesses a sequence arrangement similar to G5 except for containing one (GGA:TCC)_{12} repeat array (see Fig. 4, A and B). Such differences in arrangement may influence DNA/DNA pairing. To know whether this is the case, we examined the pairing at different combinations of probe and carrier DNA. 32P-Labeled L (A), G5 (B), and TCE (C) were incubated with 100 nM non-labeled three DNA fragments indicated below each lane. GST-HMG1AB (500 nM) was added only for A. − indicates no addition of non-labeled ds-DNA. The complexes were analyzed with 5% PAGE for A and B and with 4% PAGE for C.

Promotion of DNA-DNA Complex Formation by Histone H1—The effect of several other DNA-binding proteins on the DNA pairing was examined. Histones are major proteins abundant in the cell nucleus, and cytochrome c and single strand DNA binding protein are known to assist various DNA transactions. Among these proteins, histone H1 effectively promoted the
association of ds-DNAs containing (GGA:TCC)₁₁ repeat (Fig. 6A). However, note that there was a slight difference in the enhancing efficiency of DNA pairing between GST-HMG1AB and native histone H1; histone H1 showed an activity stronger than GST-HMG1AB, when non-labeled ds-DNA was absent in the reaction mixture. Also, GST fusion protein of histone H1 (GST-histone H1) containing the whole 211 amino acid residues was synthesized and examined. As shown in Fig. 6B, GST-histone H1 exhibited promotion activity for the complex formation, and the level of activity was similar to that of native histone H1. These results indicated that HMG1 protein and histone H1 both have promotion activity of DNA pairing.

**Distamycin and Actinomycin D Inhibit DNA Pairing Promoted by HMG1 and Histone H1**—Distamycin and actinomycin D are antibiotics that are known to bind in the minor groove of DNA with strong preferences for adenine/thymine (A:T) pair stretches and for guanine/cytosine (G:C) base pairing, respectively (30–34). The effect of the two drugs on the complex formation was examined using ³²P-labeled G5-DNA. The two different conditions used for assay of DNA pairing were as follows: 1 nM probe DNA concentration and the presence of 100 nM non-labeled ds-M2 in the presence (indicated as +) and absence (indicated as −) of 1 μM various proteins indicated below each lane. The proteins included GST fusion HMG1AB, histones H1, H3, and H4, cytochrome c (Cyt.C), and E. coli single strand binding protein (SSB). After proteinase K treatment (75 μg/ml) at 4 °C for 2 h, reaction mixtures were run on 5% PAGE. B. ³²P-labeled ds-L was incubated with (indicated as +) and without (indicated as −) 100 nM non-labeled ds-M1 in the presence (indicated as +) and absence (indicated as −) of 500 nM protein of GST, GST-HMG1AB, or GST-histone H1 as indicated.

**DISCUSSION**

**Pairing between DNAs Containing (GGA:TCC)ₙ Repeats**—In this study we show an ability of DNAs containing (GGA:TCC)ₙ repeats to pair with each other, by demonstrating a gel mobility shifted band on gels when DNA fragments containing the repeats are incubated in the presence of HMG1 or histone H1 (Figs. 1 and 6). The pairing is influenced by the concentrations of non-labeled carrier DNA and proteins of GST-HMG1 and histone H1 (Fig. 3). The structure of the complex is investigated by comparison of its mobility to those of four synthetic markers, a heterodimer, a heterotrimer, and heterotetramers (Fig. 2). A similar mobility is given between the complex and the heterotetramer markers, suggesting that it consists of two DNA molecules paired probably at the GGA repeat stretches. Examination of dissociation of the DNA-DNA complex reveals that the complex is stable up to 60 °C (Fig. 3D). Such pairing is not restricted to synthetic DNAs but is observed in genomic DNAs. G5 and TCE sequences harboring GGA repeats, although their repeat sequence is imperfect and degenerates, exhibit the complex formation (Fig. 4). Interestingly, this assembly occurs at a 100 nM DNA concentration even in the absence of GST-HMG1 protein. This suggests that the efficiency of DNA pairing is probably affected by the repeat length or may be modulated by sequences surrounding the (GGA:TCC)ₙ repeats. In fact, DNA/ DNA pairing has been detected only between DNAs containing homologous sequences under the assay conditions used. Intriguingly, DNA fragment containing synthetic (GGA:TCC)₁₁ failed to pair with TCE DNA fragment that comprised (GGA: TCC)₁₁ and vice versa (Fig. 5). Although details of this sequence dependence have not been investigated yet, it suggests that pairing between two fragments containing (GGA:TCC)ₙ repeats may require conformations to suit each other.

The pairing probably occurs at the repeat sequence of (GGA: TCC)ₙ, since pUC118 polylinker sequence does not form DNA/ DNA conformers under the same assay condition (data not shown). Association of the complex is probably due to G:G base pairing between the two GGA repeats, because our previous experiment showed that d(GGA)₁₁ oligonucleotides, but not d(GAA)₁₁, form homoduplex in a parallel orientation (6). The finding that the homoduplex formation between d(GGA)₁₁ oligonucleotides is not inhibited by complete methylation at N-7 of guanine residues of the oligonucleotides given by dimethyl sulfate treatment suggests the association through the pairing of N-1 and O-6 of guanine. Modification of ³²P-labeled ds-DNA containing (GGA:TCC)₁₁ repeat did not give any difference in DNA assembly (data not shown). This suggests that the assembly may be formed by the same base pairing.

**Promotion of DNA Pairing by HMG1 and Histone H1**—Distamycin recognizes AT-rich stretches by interacting with the minor groove of DNA (31); each of four NH groups of distamycin is hydrogen-bonded to two base pairs of the N-3 of adenine and O-2 of thymine. The presence of 1 μM distamycin in the incubation mixture inhibits DNA pairing with the aid of HMG1 or histone H1 (Fig. 7, A and B). The binding of distamycin to DNA is known to affect the activities of topoisomerases I and II (32, 33) and RNA polymerase II (34) probably by inducing alteration of DNA secondary structure. The concentration of distamycin required for this effect is similar to that of the DNA pairing promoted by HMG1 and histone H1 (32, 34). These results suggest that the binding of this antibiotic affects interaction between proteins of HMG1 and histone H1 and minor groove of DNA which may enhance G-G pairing. This interpretation is consistent with HMG1 that contacts DNA through the minor groove. However, it is known that histone H1 interacts primarily with the major groove although not exclusively (25). The mechanism for its inhibitory effect on the enhancement of...
Protein-DNA complexes were digested followed by incubation at 37 °C for 1 h. 5% PAGE was added to each reaction mixture and analyzed.

**Histone H1 and Histone H1 Proteins Both Affect DNA Pairing**

The HMG proteins belong to a family that possesses a DNA binding motif called the HMG box, which is shared by abundant non-histone components of chromatin and by specific regulators of transcription and cell differentiation (13). HMG1 and HMG2 are present in a ratio of 0.2–0.3 molecules each per nucleosome in rat tissues (10) and in 0.05 molecules in rabbit thymus cells and chicken erythrocytes (8, 9). They bind to the minor groove of double-stranded and single-stranded DNA with no apparent sequence specificity (35), but they prefer to bind to irregular DNA structures like four-way junction and cruciform DNAs (14) and cis-platin-modified DNA (17). Histone H1 also prefers to bind to four-way junction DNA (36), and hence the two proteins are suggested to have shared functional roles in organizing linker DNA in the nucleosome (22, 37, 38). It is not surprising, therefore, that HMG1 and histone H1 both promoted the DNA association. Interestingly, it is reported that HMG-D, the *Drosophila melanogaster* homologue of HMG1 protein, may function in reorganization of chromatin (39). During the earliest phases of *Drosophila* embryogenesis, condensed chromatin structures are associated with HMG-D but lack histone H1. With the appearance of histone H1, chromatin becomes progressively more compact until eventually HMG-D is supplanted by the histone (39). Since histone H1 showed a stronger activity to enhance the DNA pairing than HMG1 (Fig. 6B), the two abundant chromosomal proteins might be involved in alternative modes of chromatin compaction.

Histone H1 is known to be phosphorylated in a cell cycle-dependent manner, suggesting that the phosphorylation plays a role in the cell cycle regulation (37). GST fusion histone H1 expressed in *E. coli*, thereby not properly phosphorylated, promoted the DNA-DNA complex formation at a level similar to native histone H1 (Fig. 6B). This suggests that phosphorylation of histone H1 may not play a major role in the promotion activity. However, it is necessary to clarify more precisely whether or not phosphorylation of histone H1 influences the DNA pairing.

**Biological Roles of Repetitive Sequences Able to Associate**

DNA pairs comprising two ds-DNAs containing GGA:TCC repeats exhibit some sequence dependence but could take place among many chromosomal regions, because such repeats are abundant in the nucleus (5). It is possible that those complexes act as architectural elements constituting chromosomal domains or providing condensation (or decondensation) of the 30-nm fiber. This may be of particular importance during times of chromatin remodeling in cells undergoing DNA replication and mitosis. HMG1/2 and possibly histone H1 might not only facilitate but maintain such higher order chromatin structures (13, 20, 21, 37, 40, 41). Such promotion activity of HMG1/2 and histone H1 is analogous to the function of certain protein chaperones, which stabilize a polypeptide in a conformation that is appropriate for subsequent assembly, but would be unstable without a chaperone (41). The abundance of HMG1/2 and histone H1 in the nucleus is consistent with a general requirement for those conformations.

HMG1/2 proteins, but not histone H1, have another activity of DNA binding that can be induced by their binding (13, 18, 37, 42). The bending is also supposed to confer conformational changes of chromatin, which may help various proteins to bind to DNA, loop DNA to allow protein-protein interactions that bind to distant DNA-binding sites, or mediate the positioning of nucleosomes. The difference in the two proteins might be important as concerns different levels of expression during development mentioned above.

There are other tandem repeats that can form four-stranded DNA complexes: telomeric DNA (43–45), guanine-rich sequences (46), and (CA:TG) repeats (47). HMG1 preferentially binds to the latter repeats (47, 48). Those four-stranded DNA complexes also could function as architectural elements in the nucleus. Recently, Csík and Henikoff (49) and Dernburg *et al.* (50) reported that the insertion of heterochromatin block consisting of (AAGAG:CTCTT) repeats into the coding region of the brown eye locus (euchromatin) of *D. melanogaster* results in a position-effect variegation. Its homologous copy migrates to the centromeric heterochromatin region containing the same (AAGAG:CTCTT) repeats and undergoes transcriptional silencing. This evidence leads us to imagine that the sequence-specific association between (AAGAG:CTCTT) repeats like (GGA:TCC) repeats is involved in the association between the inserted brown eye locus and the centromeric heterochromatin. This implication is supported by the result that DNA containing the (AAGAG:CTCTT) repeat forms DNA pairing with the same association assay described here.2

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