

## DNA-Binding Property of the Novel DNA-Binding Domain STPR in FMBP-1 of the Silkworm *Bombyx mori*

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The STPR domain is a novel DNA-binding domain composed of repeats of 23 amino-acid-long peptide found in the fibroin-modulator-binding protein-1 (FMBP-1) of the silkworm *Bombyx mori*. Theoretical proteins having the STPR domain are highly conserved, particularly in vertebrates, but the functions are mostly unknown. In this study, the DNA-binding property of the STPR domain in FMBP-1 was examined. Use of reagents selecting the DNA groove and an oligonucleotide in which the dA:dT pairs of the probe were replaced with dI:dC pairs in mobility shift assay demonstrated that FMBP-1 approaches DNA from the major groove. Permutation electrophoresis using probes of the same length but containing the FMBP-1-binding site at different positions showed that FMBP-1 bends DNA through its binding. To induce the sharp bend of DNA, the STPR domain alone was insufficient and the long N-terminal extending region was necessary. Moreover, the basic region extending from the N-terminus of the STPR domain stabilized the DNA binding of the STPR domain. These results suggested that DNA-binding properties of the STPR domain are affected strongly by the structure of the flanking regions in the STPR domain-containing proteins.

**Key words:** *Bombyx mori*, DNA bending, *fibroin gene*, FMBP-1, STPR domain.

Abbreviations: CFR, C-terminal flanking region; DTT, dithiothreitol; FMBP-1, fibroin-modulator-binding protein-1; GST, glutathione-S-transferase; MSG, middle silk gland; NFR, N-terminal flanking region; NP40, nonidet P-40; PSG, posterior silk gland; STPR, score and three amino-acid-long peptide repeat; TFA, trifluoroacetic acid.

Fibroin-modulator-binding protein-1 (FMBP-1) binds the upstream and intronic promoter elements of the fibroin gene in the silkworm *Bombyx mori* (1–5). The DNA-binding activity of FMBP-1 occurs in a tissue- and stage-specific manner in conjunction with fibroin gene expression, but FMBP-1 itself is expressed in the posterior silk glands (PSG) at the fourth molt stage and the middle silk glands (MSG) (5), when and where the fibroin gene is not expressed. The DNA-binding activity of FMBP-1 appears to be controlled post-transcriptionally through protein modifications and/or interactions with other factors.

To understand the mechanism regulating the activity of FMBP-1, it is important to characterize the DNA-binding properties of FMBP-1. FMBP-1 contains four tandem repeats of the score and three amino-acid-long peptide (STP) as a DNA-binding domain in the C-terminal half of the protein (5). Each repeat of STPR is remarkably homologous and forms a similar 3D structure, but seems to have different roles in DNA binding (6, 7). The repeat structure of STP is conserved in at least *C. elegans*, *D. melanogaster* and vertebrates, but the functions of the genes encoding STPR-containing proteins remain mostly unknown.

In this study we have demonstrated that FMBP-1 binds to DNA from the major groove and bends DNA through its binding. Using FMBP-1 deletion mutants, effects of the flanking regions on the DNA binding of STPR were examined. The N-terminal basic region extending from the STPR domain stabilized the DNA binding of FMBP-1 to DNA.

### MATERIALS AND METHODS

**Preparation of FMBP-1 Proteins**—GST-fused FMBP-1 was prepared as described (5). For separation of the GST-tag from the GST-fused proteins, Sepharose beads bound with the fusion protein were washed with proteolysis buffer (50 mM Tris-HCl, pH 7.0, 150 mM NaCl, 1 mM EDTA and 1 mM DTT), and resuspended in a small amount of the same proteolysis buffer. PreScission protease (60 units/ml beads) was added and mixed with rotation overnight at 4°C. The mixture was centrifuged, and the protein separated from GST in the supernatant was collected and dialysed against NP40 buffer (50 mM Tris-HCl, pH 7.9, 12.5 mM MgCl<sub>2</sub>, 100 mM NaCl, 0.1 mM EDTA, 20% glycerol, 0.1% NP40 and 1 mM DTT). Structures of the deletion proteins are described in Fig. 5A. POU-M1 was prepared with the same methods.

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The preparation of proteins without any tag was carried out as described (7). Crude extract was loaded onto a SP-Sepharose column, and fractionated with a linear gradient from 0 M to 1 M NaCl. The peak fractions were pooled and refractionated by reverse-phase HPLC. TFA was added to the eluate from the SP-Sepharose column, and the pH was adjusted to below 4.0. Samples were loaded on a reverse-phase HPLC column, and the proteins were eluted with a 0–40% linear gradient of acetonitrile in 0.1% TFA. The peak fractions were pooled, lyophilized, redissolved in phosphate-buffered saline, and diluted with NP40 buffer.

**Electrophoretic Mobility Shift Assay**—Electrophoretic mobility shift assays were carried out as described by Takiya *et al.* (4). The oligonucleotide probe +290 (Fig. 3) (0.4–0.5 ng) was incubated with an appropriate amount of recombinant FMBP-1 protein in 10  $\mu$ l of reaction mixture (30 mM Tris-HCl, pH 7.9, 7.5 mM MgCl<sub>2</sub>, 60 mM NaCl, 0.1  $\mu$ g of poly(dI-dC), 20% glycerol and 0.06% NP40). In the experiments shown in Fig. 7, 110  $\mu$ l of reaction mixture was prepared for the binding step before addition of the competitor. The protein–probe complexes were analysed by electrophoresis on gels with 6–11% polyacrylamide. The intensity of shifted bands was measured with a BAS2000 bioimage analyzer (Fuji Film).

Probes having the same length but containing the FMBP-1 recognition sequence at different positions for pseudopermutation electrophoresis were prepared with pBluescript. The +290 oligonucleotide was inserted into the EcoRI site and 147 bp DNA fragments (A to G in Fig. 4A) were amplified by PCR with the following primer sets: A (5' CAGTCACGACGTTGTAAAC3' and 5' TGAATCTATGTAAATACTGGGC3'), B (5' CGGCCAGTGAATTGTAATAC3' and 5' GATAAGCTTGATATCGAATTGAT3'), C (5' CTCACATATAGGGCGAATTG3' and 5' CCCCCTCGAGGTCGAC3'), D (5' CTCACCGCGGTGGCGG3' and 5' AACAAAAGCTGGGTACCG3'), E (5' TAGAACTAGTGGATCCCC3' and 5' GCAATTAACCTCACTAAAGG3'), F (5' CTGCAGGAATTGTCTGCC3' and 5' TGACCATGATTACGCCAAG3') and G (5' ATTTACATAGATTCATCAATTTCGATAT3' and 5' AATTTACAGGAAACAGCTA3'). Using the primers for probe D, two different sizes of DNA were amplified. The 147-bp PCR amplicons were purified by acrylamide gel electrophoresis and used for labeling. The probes of permutation analyses were labelled with [ $\gamma$ -<sup>32</sup>P]ATP and T4 polynucleotide kinase.

Distamycin A, methyl green and netropsin were purchased from Sigma-Aldrich Co., and Hoechst 33258 was purchased from Wako Chemical Co.

## RESULTS

**STPR Proteins are Highly Conserved in Vertebrates**—The STPR domain first found in the FMBP-1 protein of the silkworm *Bombyx mori* (5) is a novel DNA-binding domain composed of repeats of the score and three amino-acid-long peptide (Fig. 1). The homology between each repeat in the STPR domain of FMBP-1 is extremely high. We found genomic and/or cDNA sequences encoding theoretical proteins having four tandem repeats of 23 amino acids homologous to the STPR of FMBP-1 in a

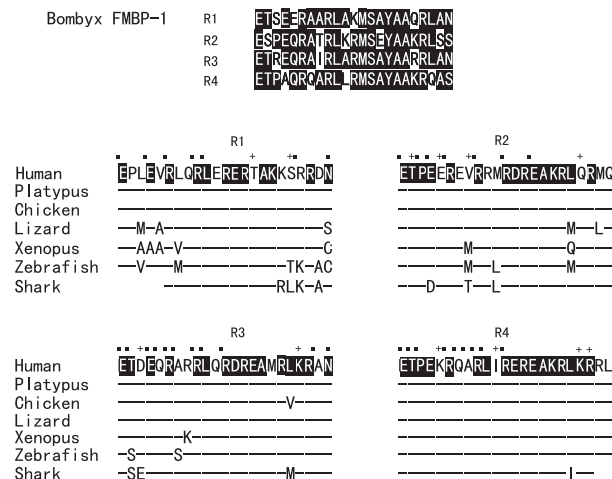


Fig. 1. Conservation of the STPR domain in vertebrates. Amino-acid sequence of the STPR in FMBP-1 of the silkworm and various vertebrates. Amino acids indicated with white letters on black appeared more than twice at the same position of each repeat. The amino acids indicated with a dot or + are identical or similar to the amino acids of the corresponding positions of FMBP-1. R1; repeat-1, R2; repeat-2, R3; repeat-3, R4; repeat-4. Platypus; *Ornithorhynchus anatinus*, Lizard; *Anolis carolinensis*, Xenopus; *Xenopus tropicalis*, Zebrafish; *Danio rerio*, Shark; *Callorhynchus milli*.

wide variety of vertebrates as shown in Fig. 1. Amino-acid sequences of the STPR domains are conserved from sharks to humans, and the homology between the repeats is high as in FMBP-1. Furthermore, regions outside of the STPR domain in theoretical proteins are also conserved in vertebrates (data not shown), although no homology in these regions was found between FMBP-1 and the identified theoretical proteins. These findings suggest that proteins with STPR domains play important roles in animals.

**Effects of DNA-Groove-Selecting Reagents on the Binding of FMBP-1**—FMBP-1 binds an AT-rich DNA element (Fig. 3), and some minor groove-binding proteins bind to AT-rich sequences (8, 9). To explore the DNA-binding property of FMBP-1, we first used groove-selecting reagents in mobility shift assay. Netropsin, Hoechst 33258 and distamycin bind specifically to the minor groove of DNA (10, 11) and inhibit the binding of minor groove-binding proteins. Methyl green binds specifically to the major groove (12) and inhibits the binding of major groove-binding proteins. As shown in Fig. 2A, up to 1 mM of netropsin and 100  $\mu$ M of Hoechst 33258 did not inhibit the binding of FMBP-1. Because more than 100  $\mu$ M of Hoechst affected the mobility of the free probe per se, it became difficult to distinguish between the shifted band and free probe.

The FMBP-1-recognition sequence is composed of dA:dT pairs alone (ATNTWTNTA) (Fig. 3), and all the minor groove-selecting reagents used here prefer AT-rich regions, whereas the major groove-selecting reagent methyl green prefers GC-rich regions. Therefore, we examined effects of methyl green at up to 1 mM. As shown in Fig. 2B, 0.5 and 1 mM of methyl green tended to inhibit the binding of FMBP-1. These results

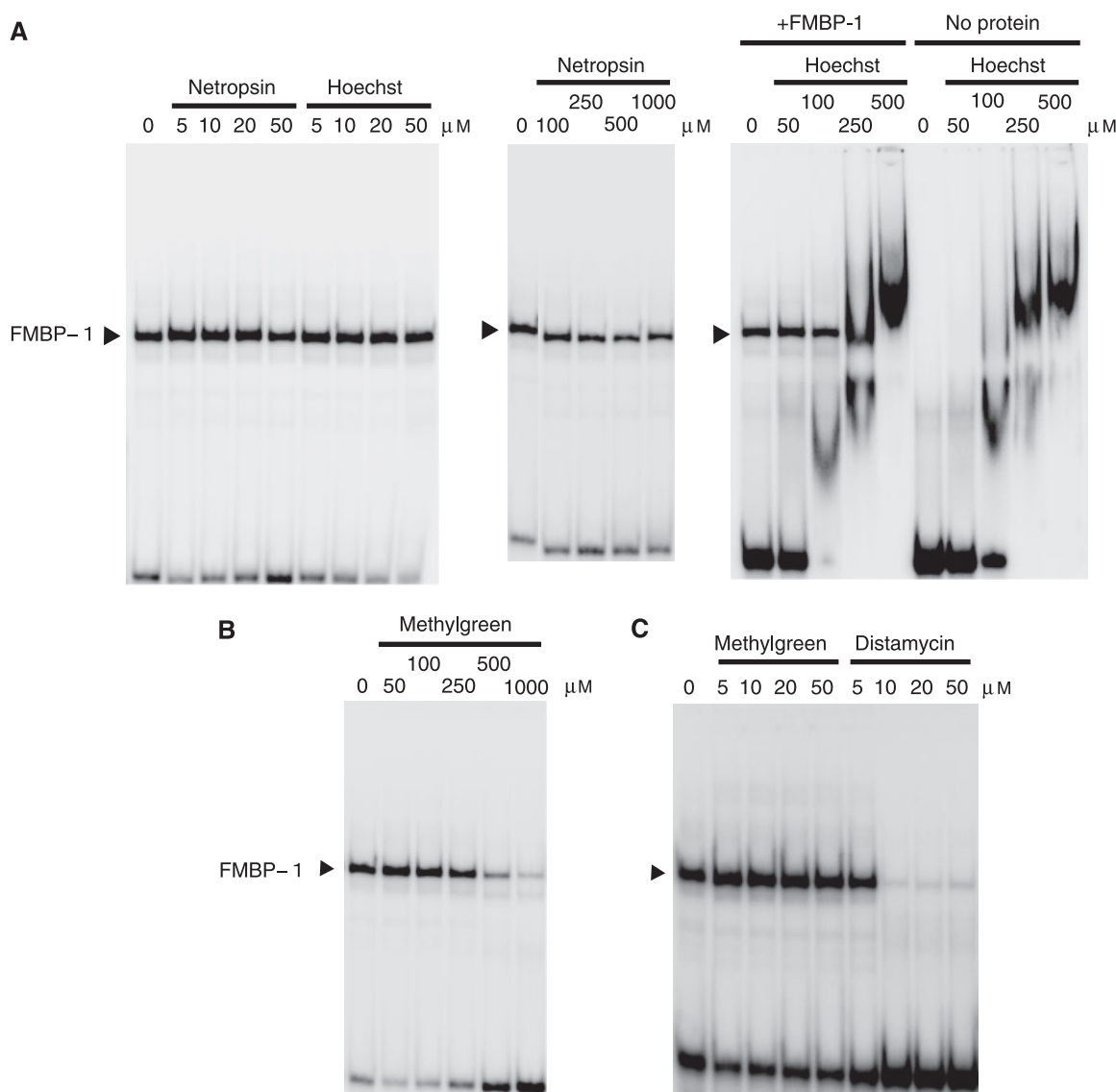


Fig. 2. **Major groove binding of the STPR domain.** GST-free FMBP-1 protein (FMBP-1) was prepared from GST-fusion proteins by treatment with PreScission protease. Each protein (200 fmol) was incubated with the +290 oligonucleotide probe in

the presence or absence of Netropsin (0–1000  $\mu\text{M}$ ), Hoechst 33258 (0–500  $\mu\text{M}$ ) (A) methylgreen (0–1000  $\mu\text{M}$ ) (B) or distamycin (0–50  $\mu\text{M}$ ) (C) and analysed on a 7% polyacrylamide gel.

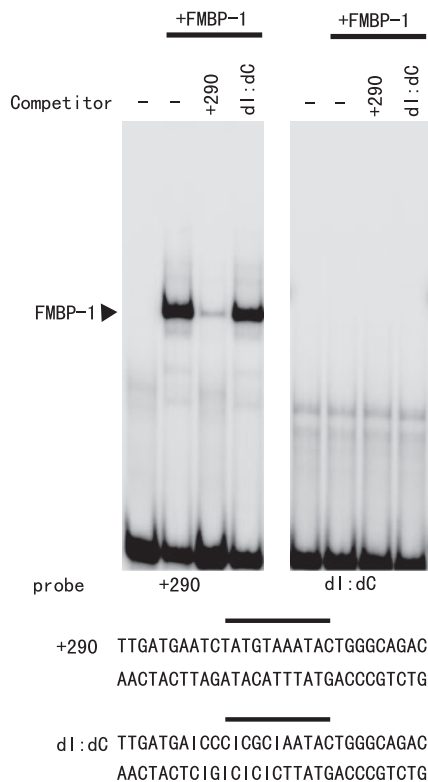
suggested that FMBP-1 bound to the major groove of DNA.

Distamycin gave an apparently controversial result (Fig. 2C). Distamycin binds selectively to the minor groove of DNA (10), but inhibited the binding of FMBP-1 at the lowest concentration (10  $\mu\text{M}$ ) of the reagents used. No inhibitory effect by methyl green on the FMBP-1 binding was observed by at such low concentrations. The inhibition of some major groove binding-proteins by distamycin was reported, because of a conformational change induced by distamycin bound to the minor groove (13).

**Replacing dA:dT Pairs with dI:dC Pairs in the Sequence Recognized by FMBP-1 Inhibits the Binding of FMBP-1**—The minor grooves of dA:dT pairs and dI:dC pairs of DNA are identical in the structure.

Therefore, proteins that bind to the minor groove will not be affected by the replacement of dA:dT pairs with dI:dC pairs in the binding element. We prepared a +290(dI:dC) oligonucleotide in which dA:dT pairs of the FMBP-1-recognition sequence of the +290 probe were replaced with dI:dC pairs (Fig. 3), and used for EMSA as the probe and competitor. As shown in Fig. 3, the +290(dI:dC) oligonucleotide did not compete with the binding of FMBP-1 to the +290 probe at all, and we did not detect any binding of FMBP-1 as a probe. Thus, all the above results demonstrated that FMBP-1 approaches DNA from the major groove, but not from the minor groove.

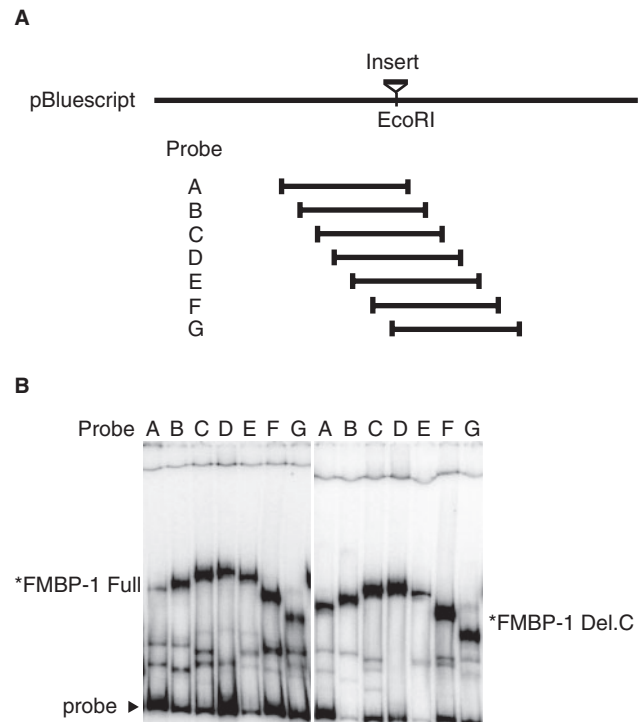
**FMBP-1 Bends DNA Through its Binding**—To assess the bending of DNA by FMBP-1, the +290 oligonucleotide usually used as a probe for FMBP-1 in the mobility shift



**Fig. 3. Inhibition of FMBP-1 binding by the replacement of dA:dT pairs of the probe with dI:dC pairs.** dA:dT pairs in the FMBP-1-recognition element of the +290 probe were replaced with dI:dC pairs as shown below the panels and used as the probe and competitors in EMSA. The position of the FMBP-1-recognition element is indicated with a line on the nucleotide sequence.

assay was inserted into the EcoRI site of pBluescript and a series of 147-bp DNA fragments were amplified by PCR with primer sets targeting shifted positions in the pBluescript clone (Fig. 4A). These fragments were used as probes for mobility shift assay. The mobility of complexes of FMBP-1 and these probes was altered depending on the position of the FMBP-1-binding site (Fig. 4B). Complexes with probes having the binding site at the center (probe D) were most retarded. Other complexes migrated faster as the binding site moved to the ends of the probes, though the complexes containing probe A tended to move more slowly than those with probe G. These shifted patterns suggested that DNA bending was induced by FMBP-1 (14–18). The bend angle was calculated simply by comparing the mobility of the complexes with probes D and G (mobility of the probe D complex/mobility of the probe G complex =  $\cos \alpha/2$ ) (19) from the results shown in Fig. 3B. The angle was about 90° for both GST-free proteins, \*FMBP-1 (asterisk indicates where the FMBP-1 and tags are joined, Fig. 5A) and \*FMBP-1 Del.C lacking the C-terminal region, though the calculated angles were altered depending on the gel concentrations used for analyses (Fig. 5C).

*The STPR Alone is Insufficient for the Sharp Bending of DNA*—To examine effects of the hyperbasic N-terminal



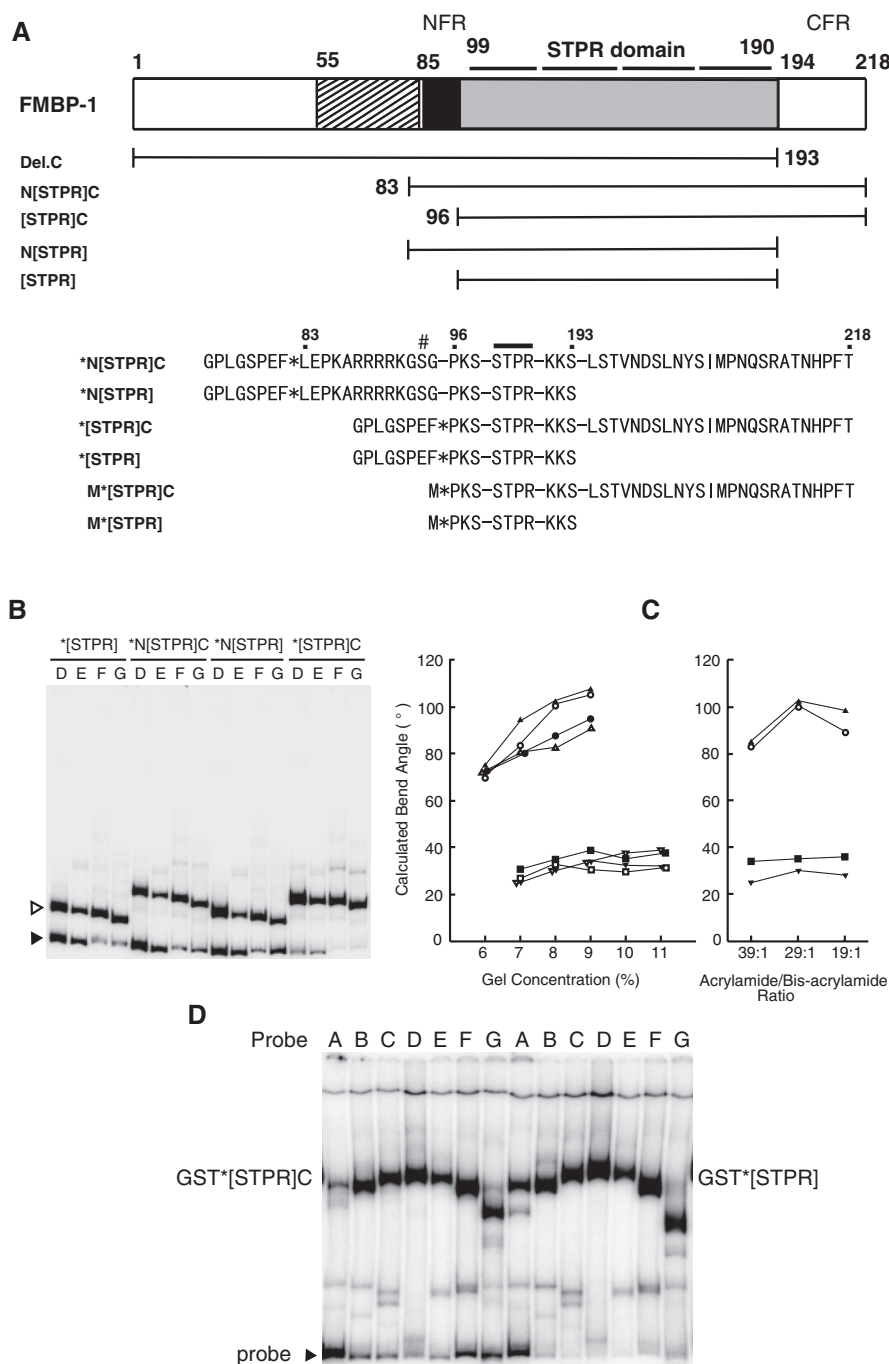
**Fig. 4. FMBP-1 bends DNA through its binding.** (A) The +290 oligonucleotide was inserted into the EcoRI site of pBluescript, and 147 bp of DNA (A–G) were amplified by PCR with appropriate primer sets. After phenol–chloroform treatment and ethanol precipitation, a portion of the PCR mixture was used directly for labeling with  $[\gamma\text{-}^{32}\text{P}]\text{ATP}$  except for primer set D. (B) Pseudopermutation electrophoresis of GST-free FMBP-1 (\*FMBP-1 Full) and FMBP-1 lacking CFR (\*FMBP-1 Del.C).

flanking region (NFR) and the C-terminal flanking region (CFR) of STPR on DNA binding, we prepared proteins containing the STPR domain with various extended regions (Fig. 5A): GST-free STPR (\*[STPR], \*[STPR]C, \*N[STPR] and \*N[STPR]C), and STPR without any tag (M\*[STPR] and M\*[STPR]C). These proteins were tested for their ability to bend DNA. Proteins with short extended regions induced only slight bending ( $\sim 30^\circ$ ) irrespective of their structure (Fig. 5B).

We further analysed the DNA-bending induced by these proteins with gels containing different concentrations of polyacrylamide. As shown in Fig. 5C, the angles calculated from the mobility in the different gels varied, but under all conditions used in this study, the STPR proteins with short extended regions did not bend DNA as sharply as the intact FMBP-1. However, we found that the STPR fused to GST at the N-terminus gave a similar pattern to the complete FMBP-1 in the permutation electrophoresis (Fig. 5D). Therefore, to bend DNA sharply, the long N-terminal extended region was necessary. A possible role of the long N-terminal extended region in the bending is discussed in the DISCUSSION section.

*The Basic N-Terminal Extended Region of STPR Stabilized the STPR–DNA Complex*—Effects of the extended regions on the stability of the STPR–DNA





**Fig. 5. Effects of the extended regions of STPR on DNA bending.** (A) The oblong box shows the structure of FMBP-1. The box with diagonal lines indicates the acidic region, the black box indicates the hyperbasic NFR, the gray box indicates the STPR domain, and horizontal lines above the gray box indicate repeats of the 23 amino acids. Long horizontal lines under the box indicate the region covered by each deletion protein. In the lower half of the figure, amino-acid sequences of the flanking regions of the STPR domain are shown. PKS-STPR-KKS corresponds to the gray box. The line over the STPR indicates the four tandem repeats of 23 amino acids. Asterisks indicate the positions where GST and FMBP-1 were joined, or where the initiating methionine was joined to FMBP-1. Hyphens indicate where NFR and the STPR domain were joined, or where the STPR domain and CFR were joined. The sharp shows a possible phosphorylation site in the hyperbasic NFR. N and C in

the clone or protein names indicate the presence of the NFR and CFR, respectively. (B) Probes shown in A of Fig. 4 were incubated with the GST-free proteins \*N[STPR], \*N[STPR]C, \*N[STPR]C and \*N[STPR]C. Closed triangles show probes, and open triangles show shifted bands. (C) Calculated bending angles induced by STPR proteins. GST\*[STPR]; closed triangle, GST\*[STPR]C; open triangle, \*FMBP-1; open circle, \*FMBP-1 delC; closed circle, \*N[STPR]C; reversed open triangle, \*N[STPR]C; closed square, \*N[STPR]; reversed closed triangle and \*N[STPR]; open square, were used for EMSA and analysed on gels with different concentrations (6–11%; acrylamide:bisacrylamide=29:1, or 8%; acrylamide:bisacrylamide=39:1, 29:1 or 19:1). The average of three experiments for each point is indicated. (D) The STPR domains with a GST-tag were used for pseudopermutation analyses.

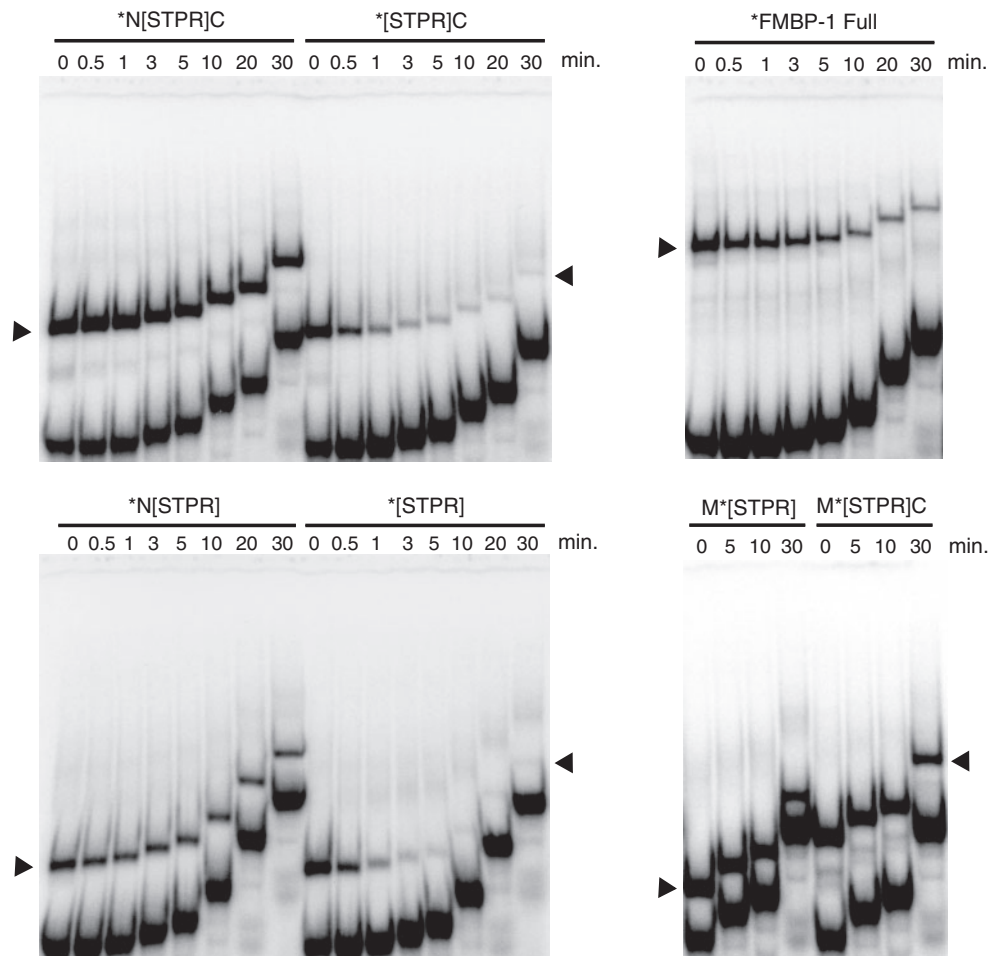


Fig. 6. **Stability of FMBP-1-DNA complexes.** After the binding reaction (110  $\mu$ l), a 100-fold amount of the unlabelled + 290 oligonucleotide was added to the reaction mixture. A portion (10  $\mu$ l) of the mixture was withdrawn at each time point after the competitor was added indicated at the top of the panels and

analysed on 10% polyacrylamide gels. For the proteins (\*N[STPR]C, \*[STPR]C, \*N[STPR], \*[STPR] and \*FMBP-1 Full) prepared by protease digestion, the concentration was adjusted to 20 nM, and the no-tag protein (M\*[STPR]C and M\*[STPR]) was adjusted to 4 nM.

complex were analysed using competitor challenge experiments after the binding reaction (see MATERIALS AND METHODS section, and also Fig. 6). As shown in Fig. 6, the structure of the N-terminal extended region of the STPR strongly affected the stability of the STPR-DNA complex. GST-free \*N[STPR]C and \*N[STPR] with the hyperbasic NFR formed stable complexes (80.3% and 87.3% preserved, respectively, 5 min after the competitor was added, and 75.8% and 70.3% even after 30 min). Whereas complexes of GST-free \*[STPR]C and \*[STPR] lacking NFR were unstable. When the hyperbasic NFR was linked to the STPR, CFR did not contribute to the stability, however when the NFR was excluded, the CFR somewhat stabilized the STPR-DNA complex. The rate of preservation was 14.5% for \*[STPR]C having CFR and 5.1% for \*[STPR] lacking CFR 5 min after the competitor was added. The stability of complexes with no-tag proteins was relatively high, even though those proteins lacked the hyperbasic NFR. About 85% of the complexes were maintained at 5 min after the challenge, and then the amount of complexes decreased gradually. The rate

of preservation of the entire FMBP-1 protein was 43.1% 5 min after the challenge and 17.4% after 30 min. Therefore, the structures of both extended STPR regions affect the stability of STPR-DNA complexes, but this effect appears to be reduced in the entire FMBP-1 protein.

**FMBP-1 can Bind to DNA as a Monomer**—The leucine zipper-type transcription factors have a basic region on the N-terminal side of their DNA-binding domain, form homo- and hetero-dimers, and bind to DNA. We examined whether FMBP-1 binds DNA as a monomer or dimer, though it is known that STPR can bind to DNA as a monomer (7). First, we compared intensities of bands obtained under increasing protein concentrations of GST\*FMBP-1, \*FMBP-1 and \*STPR in mobility shift assay. As shown in Fig. 7A, no new band appeared, and the ratio of band intensities detected at a low protein concentration was not changed even at high concentrations. Thus, the DNA binding form of FMBP-1 was not changed from monomer to dimer by the increasing protein concentrations. We detected a complex either

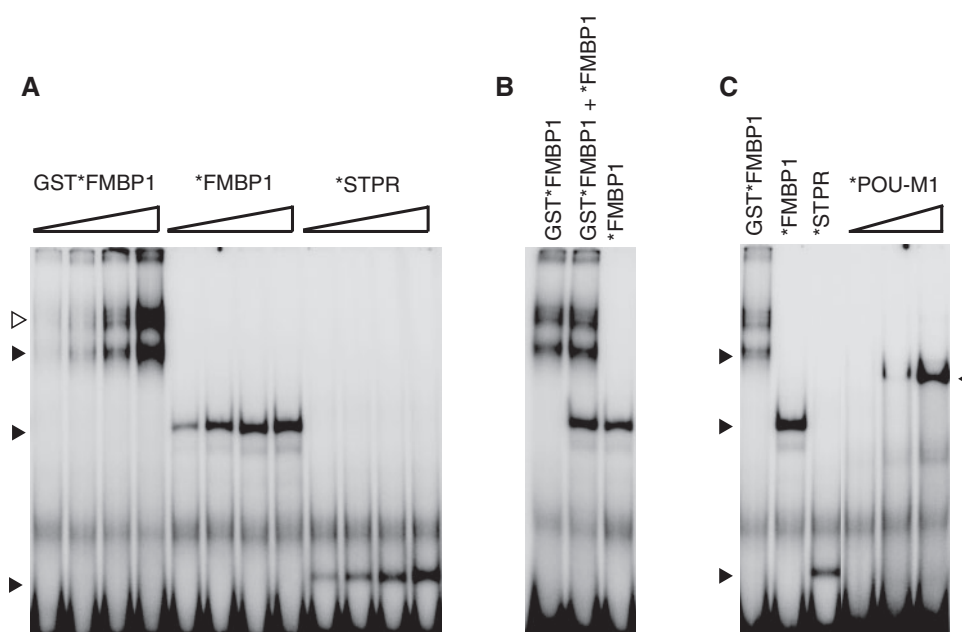


Fig. 7. **FMBP-1 binds to DNA as a monomer.** (A) Increasing amount (50, 100, 200 and 400 fmol) of the proteins (GST\*FMBP-1, \*FMBP-1 and \*STPR) were used for mobility shift assay with the +290 probe. (B) GST\*FMBP-1 and \*FMBP-1 proteins were

mixed and used for mobility shift assay. (C) Mobility of the complexes with GST\*FMBP-1, \*FMBP-1 or \*STPR was compared with that of the complex containing \*POU-M1 with the +290 probe.

with the monomer or dimer of FMBP-1 alone. With GST-fused FMBP-1 (GST\*FMBP-1), low mobility bands seemed to be the dimer and discontinuous constraint forms via the GST-tag were observed.

The mixing of GST\*FMBP-1 and \*FMBP-1 proteins did not result in any intermediate sized complex (Fig. 7B), indicating that FMBP-1 binds to DNA as a monomer, or no exchange of the components of the dimer occurred. Next we compared the mobility of the complex of FMBP-1 (25 kDa) with that of POU-M1 (39 kDa) (20). The +290 oligonucleotide probe contains a POU-homeodomain-binding element and a forkhead-domain binding element as well as the FMBP-1-binding element (4). The complex with FMBP-1 moved faster than the complex with POU-M1 on the same gel using the same probe (Fig. 7C). It is known that BmFkh (39 kDa) (18) gives a shifted band at almost the same position as POU-M1 (4). These results demonstrated that FMBP-1 did not form a dimer like leucine zipper transcription factors and bound to DNA as a monomer.

## DISCUSSION

**FMBP-1 Approaches DNA from the Major Groove**—The minor groove-selecting reagent distamycin inhibited the DNA binding of FMBP-1 and the major groove-selecting reagent methyl green did not inhibit the DNA binding at low concentrations. However, other minor groove-selecting reagents netropsin and Hechst 33258, did not inhibit the DNA binding of FMBP-1 even at high concentrations (1 mM and 100  $\mu$ M, respectively). All these minor groove-selecting reagents prefer AT-rich sequences such as the FMBP-1 recognition sequence, while methyl green

prefers GC-rich sequences. Actually, high concentrations of methyl green (at 500  $\mu$ M and 1 mM) inhibited the DNA binding of FMBP-1. Though distamycin binds to the minor groove, it inhibits some major groove-binding proteins, probably by leading a conformational change of the major groove (13). Since the structure of the minor groove of dA:dT pairs and dI:dC pairs is identical, we used the +290(dI:dC) oligonucleotide in which the dA:dT pairs of the +290 probe recognized by FMBP-1 were replaced with dI:dC pairs to explore whether FMBP-1 binds to the minor groove. The +290(dI:dC) oligonucleotide could neither compete with the binding of FMBP-1 to the +290 probe as a competitor, nor bind FMBP-1 as a probe in mobility shift assay. Thus, we concluded that FMBP-1 approaches DNA from the major groove.

**Bending of DNA Induced by FMBP-1**—The permutation gel electrophoresis suggested that FMBP-1 bends DNA. Calculated bend angles were markedly different with the complete FMBP-1 (90°) and with the STPR domain alone or STPR with short extended regions (30°). The large N-terminal region in FMBP-1 may affect the structure of the STPR domain and lead to a sharper bend. In the STPR domain alone, each repeat tends to position independently (Saito *et al.*, unpublished observations), but the long N-terminal extended region might restrict the position of each repeat. Because the artificial GST tag attached to the STPR domain could substitute for the N-terminal region of FMBP-1, there is another possibility, that the N-terminal region sticks out from the DNA axis like leucine zipper-type transcription factors and affects the mobility of the FMBP-1–DNA complex. Comparison of the mobility of the FMBP-1–DNA complex with that of complexes of other proteins of known

molecular sizes such as POU-M1 suggested that FMBP-1 did not form a leucine zipper-type dimer and bound to DNA as a monomer. To know how the complete FMBP-1 bended DNA sharply and the STPR domain alone bended DNA slightly, and whether the N-terminal half of FMBP-1 sticks out from the DNA axis, it is necessary to determine the 3D structure of the DNA-bound forms of STPR and FMBP-1.

DNA bending is involved in the transcriptional regulation of many genes. LEF-1 binds to the enhancer of the human T-cell receptor  $\alpha$  (TCR $\alpha$ ) gene and participates in the activation of its expression, but the LEF-1-binding site alone is not sufficient to stimulate the basal activity of the TCR $\alpha$  gene promoter (22, 23). LEF-1 seems to regulate interactions between other factors that bind to the enhancer through DNA bending (24). Similar results were obtained with another HMG-box protein, Sox2 (25). Moreover, the basal transcription factor TBP is well known to bind to the TATA-box and bend DNA (19, 28, 29). DNA bending at the TATA-box induced by TBP is believed to facilitate interactions between the basal transcription factors and transcriptional regulatory factors on the gene promoter. The angle produced by TBP is dependent on sequence variations of the TATA-box, and promoter activity is proportional to the angle rather than the affinity of TBP (30). FMBP-1 may regulate the fibroin gene promoter by facilitating the interaction of other transcription factors using a mechanism similar to that of those architectural transcription factors. Furthermore, FMBP-1 may act directly on the chromatin structure rather than influencing other transcription factors. Recently, the region up to  $-5$  kbp of the fibroin gene promoter was found to function as a far upstream enhancer together with the proximal promoter regions (31). In this region, many (13 or more) FMBP-1-binding sites exist. When FMBP-1 binds to all of these sites, the upstream region of the fibroin gene will become a distorted chromatin island.

**Stabilization of the STPR–DNA Complex by the Hyperbasic Region**—The hyperbasic NFR stabilized the STPR–DNA complexes, though this effect was reduced in intact FMBP-1. Similar stabilization of protein–DNA complexes by the basic extended region of the DNA-binding domain has been observed in the testes determining factor SRY (32). Replacement of NFR with the linker sequence between GST and STPR intrinsically destabilized the STPR–DNA complexes. However, more than 85% of complexes with tag-less proteins were maintained at 5 min after challenge. Therefore, the linker region seems to play an active role in the stripping of the STPR domain from DNA. In NFR, a typical protein phosphorylation site (RRKGS) exists in addition to the basic amino-acid residues. On the other hand, a glutamic acid is present in the linker region at a position that corresponds to a possible phosphorylating serine in NFR. If the replacement of this serine with glutamic acid causes the destabilization of STPR–DNA complexes, phosphorylation of the hyperbasic NFR may be involved in regulation of the DNA-binding activity of FMBP-1. It should therefore be determined whether NFR is phosphorylated in a tissue- and/or developmental stage-specific manner.

## FUNDING

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## CONFLICT OF INTEREST

None declared.

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