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Multiplicity of the DNA-binding protein HBP-1 specific to the conserved hexameric sequence ACGTCA in various plant gene promoters

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A novel DNA-binding protein that specifically interacts with the hexameric sequence ACGTCA in the regulatory region of the wheat histone H3 gene has been identified in wheat nuclear extract and designated HBP-1a. The nuclear protein HBP-1 previously identified as a DNA-binding protein that interacts with hexameric sequences in the H3, cauliflower mosaic virus (CaMV) 35 S RNA, and nopaline synthase (NOS) promoter regions therefore has been renamed HBP-1b. The flanking sequences that surround the hexameric sequence may account for the difference in the binding properties of HBP-1a and HBP-1b.

Multiplicity; DNA-binding protein; Hexameric sequence; DNA–protein interaction

1. INTRODUCTION

DNA sequences that control the transcription of the eukaryotic genes mediated by RNA polymerase II form complex arrays in the regulatory regions of individual genes [1]. Recent studies of gene expression have identified the sequence-specific DNA-binding proteins that function in transcriptional regulation [2–4]. We elsewhere reported a wheat nuclear protein HBP-1 that specifically interacts with the hexameric sequence ACGTCA and is conserved in the regulatory regions of wheat histone H3 and H4, CaMV 35 S RNA, and NOS genes [5,6] (Mikami et al., unpublished data). Results of in vivo transcription of progressive 5′-deletion mutants of H3, CaMV 35 S RNA, and NOS genes suggest that the conserved hexameric sequence may act in the transcription driven by the promoters of these genes ([7–9]; Nakayama et al., unpublished data).

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Although a number of eukaryotic transcription factors interact with a single target sequence, some factors can recognize a common sequence. They exist in multiple forms as in MLTF [10], ATF [11] and AP-1 [11], and in such closely related protein families as the CCAAT-binding proteins [12–14], octamer-binding proteins [15,16] and steroid hormone receptors [17]. We here report the multiplicity of HBP-1. Cross-competition experiments coupled with mobility shift assays showed that HBP-1 consists of at least two distinct proteins, which we have designated HBP-1a and HBP-1b.

2. MATERIALS AND METHODS

2.1. *Nuclear extracts*

Crude nuclear extracts that had been prepared from wheat germ nuclei with 0.4 M NaCl were fractionated by phosphocellulose column chromatography, as described elsewhere [5,6]. The fraction eluted with 0.6 M KCl was used in the mobility shift and competition assays [5,6] because it contained both HBP-1a and HBP-1b.

2.2. *DNA probe*

The DNA fragment of the upstream sequence that spans −274 to −130 in the wheat histone H3 gene was ^{32}P-end-

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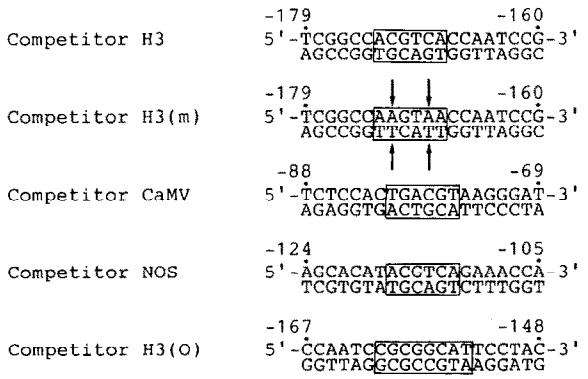


Fig.1. Sequence alignments of the synthetic double-stranded oligonucleotides used as competitors in the HBP-1 binding assay. The hexameric and octameric sequences [5,6] are boxed. Negative numbers indicate positions in the nucleotides related to the transcriptional initiation site. The competitor H3(m) carries two point mutations (arrows in the hexameric sequence). Competitors H3, H3(m) and H3(O) correspond to competitors 1, 1(m) and 2 in [6].

labeled with [α-^{32}P]dCTP and Klenow enzyme, as described elsewhere [6].

2.3. *Mobility shift assay*

Mobility shift and competition assays essentially were done by the method described elsewhere [5,6], except that electrophoresis took place in a mini-gel system (horizontal submarine type) that used Mupid-2 (Cosmo Bio Co., Tokyo). Double-stranded synthetic oligonucleotides (fig.1) were the competitors used in the mobility shift assay.

3. RESULTS AND DISCUSSION

In order to do the mobility shift assay more easily, we used a mini-gel system. The reaction mixture for this assay was divided into two equal portions which were electrophoresed in two types of gel systems. The electrophoretic patterns for the 0.7% agarose/4% polyacrylamide (composite) and 5% polyacrylamide gels are shown in fig.2. The composite gel (fig.2A) recorded a single band that migrated more slowly than the free probe (lane 2). The formation of this band was blocked by synthetic oligonucleotides with the hexameric sequences of wheat histone H3, CaMV 35 S RNA or NOS genes (lanes 3, 5 and 6), but not by oligonucleotides that contained a point-mutated hexameric or octameric sequence (lanes 4 and 7). These results are consistent with our previous findings ([5,6]; Mikami et al., unpublished data).

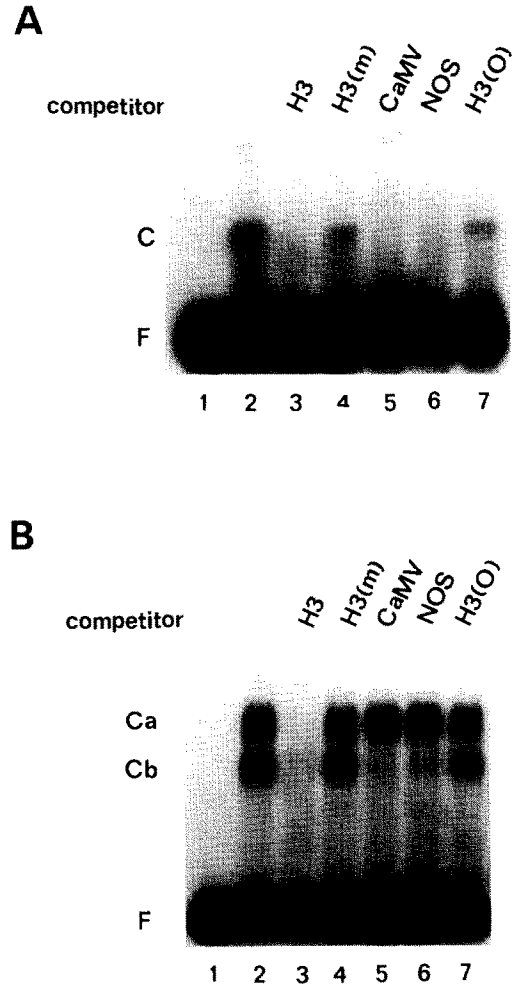


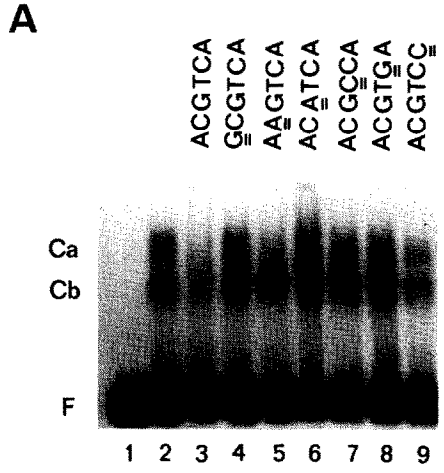
Fig.2. Electrophoretic determination of two subtypes (1a and 1b) of HBP-1 by use of a mobility shift assay. The *Xho*I-*Fok*I fragment (−274 to −130) from the wheat H3 gene was ^{32}P-end-labeled then incubated with the 0.6 M KCl phosphocellulose fraction of wheat germ nuclear extract in the absence (lane 2) or the presence (lanes 3–7) of a 50-fold molar excess of an unlabeled synthetic, double-stranded oligonucleotide (indicated above each lane), after which it was electrophoresed on low-ionic strength 0.7% agarose/4% polyacrylamide (composite) (A) or 5% polyacrylamide (B) gels. Lane 1, reaction with no protein; F, free probe; Ca, the HBP-1a-probe complex; C and Cb, the HBP-1b-probe complex.

When a 5% polyacrylamide gel was used (fig.2B), two shifted bands were recorded (lane 2). The reason for the appearance of these two bands on the 5% polyacrylamide gels is not clear. We therefore did competition experiments to examine the binding specificity of the nuclear proteins in-

volved in the formation of these DNA-protein complexes. Results showed that the fast migrating band faded out when the competitors were H3, CaMV and NOS (lanes 3, 5 and 6); whereas formation of the slowly migrating complex was prevented only by competitor H3 (lane 3). Competitors H3(m) and H3(O) had no effect on the formation of either band (lanes 4 and 7). When DNA fragments that had hexameric motifs of CaMV 35 S and NOS genes were used as probes, formation of specific DNA-protein complexes was inhibited by all the hexameric motif-bearing oligomers (data not shown). Thus, there are at least two types of HBP-1 which differ in their DNA-binding specificities. The H3 hexamer-specific binding protein responsible for the formation of the slowly migrating complex has therefore been given the tentative name HBP-1a, and the first migrating hexamer-specific binding protein the name HBP-1b. Results of cross-competition experiments ([5,6] and fig.2) indicated that HBP-1b corresponds to the nuclear protein previously designated HBP-1.

To investigate the DNA-binding specificities of HBP-1a and HBP-1b in detail, we ran competition experiments with double-stranded synthetic oligomers that contained the base-substituted hexameric sequence (fig.3). There were differences between HBP-1a and HBP-1b in their requirements for the hexameric sequence in DNA-protein binding which suggest that recognition of the ACGTCA consensus sequence by HBP-1a is less important than by HBP-1b. Moreover, the interaction between HBP-1a and the hexameric motif seems to be affected more by the flanking sequences of this motif than is the case for HBP-1b. We conclude that the DNA-binding affinities of the two nuclear proteins for the hexameric motif and its flanking sequences differ and that their DNA-binding domains also differ. The results reported here are supported by other data which show that HBP-1a and HBP-1b can be separated on a butyl-Sepharose column (Takase et al., unpublished data).

We elsewhere reported that HBP-1 does not interact with the GGCCACGTGACC sequence in the adenovirus 2 major late promoter (the binding site of a major late transcription factor, MLTF) even though the HBP-1 recognition sequence (GGCCACGTCACC) in the wheat H3 gene differs from the MLTF one by only a single nucleotide [6]. We have again obtained similar results for HBP-1b; it does not bind to the MLTF-binding site (fig.3, lane 8). Interestingly, the binding specificity of HBP-1b is very similar to that of the mammalian transcription factor ATF [18] whose recognition sequence (ACGTCA) is identical to that of HBP-1 [6]. In fact, HBP-1b could



B

	HBP-1a	HBP-1b
ACGTCA	++++	++++
GCGTCA	–	–
AAGTCA	++++	–
ACATCA	–	–
ACGCCA	+	–
ACGTGA	+	–
ACGTCC	++	++

Fig.3. Difference in the sequence specificity of the binding of HBP-1a and HBP-1b to DNA. (A) The ^{32}P-labeled probe (see fig.2) was incubated with the 0.6 M phosphocellulose fraction in the absence (lane 2) or presence (lanes 3–9) of a 50-fold molar excess of unlabeled synthetic, double-stranded oligonucleotides that bore the wild type (lane 3) or point-mutated (lanes 4–9) hexameric sequences indicated above each lane. Replaced bases are indicated by double underlines. (B) Summary of the relative binding strengths of HBP-1a and HBP-1b to the wild-type and mutated hexameric sequences.

recognize the ATF binding site in the adenovirus E4 promoter (data not shown). Hurst et al. [19] used competition assays with a series of point-mutated oligomers to examine the roles of individual nucleotides in the ACGTCA motif in ATF-binding to the hexameric motif. A comparison of the results in fig.3 with their data shows some small differences for HBP-1a, HBP-1b and ATF in their sequence requirements for binding, an indication that these three DNA-binding proteins are distinct members of a hexamer-binding protein family common to both plants and animals. If so, it is important to establish their structural and functional relationships.

Recently, we have cloned a cDNA that encodes HBP-1a (Tabata et al., unpublished data) which should prove useful for determining what the molecular mechanisms are that function in the histone gene transcription and DNA-binding specificity of HBP-1a.

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REFERENCES

[1] Breathnach, R. and Chamborn, P. (1981) Annu. Rev. Biochem. 50, 349–383.
[2] Dynan, W.S. and Tjian, R. (1985) Nature 316, 774–778.
[3] McKnight, S. and Tjian, R. (1986) Cell 46, 795–805.
[4] Maniatis, T., Goodbourn, S. and Fischer, J.A. (1987) Science 236, 1237–1244.
[5] Mikami, K., Tabata, T., Kawata, T., Nakayama, T. and Iwabuchi, M. (1987) FEBS Lett. 223, 273–278.
[6] Mikami, K., Nakayama, T., Kawata, T., Tabata, T. and Iwabuchi, M. (1989) Plant Cell Physiol. 30, 107–119.
[7] Odell, J.T., Nagy, F. and Chua, N.-H. (1985) Nature 313, 810–812.
[8] Ow, D.W., Jacobs, J.D. and Howell, S.H. (1987) Proc. Natl. Acad. Sci. USA 82, 4870–4874.
[9] An, G., Ebert, P.R., Yi, B.-Y. and Choi, C.-H. (1986) Mol. Gen. Genet. 203, 245–250.
[10] Sawadogo, M., Van Dyke, M.W., Gregor, P.D. and Roeder, R.G. (1988) J. Biol. Chem. 263, 11985–11993.
[11] Hai, T., Liu, F., Allegretto, E.A., Karin, M. and Green, M.R. (1988) Genes Dev. 2, 1216–1226.
[12] Jones, K.A., Yamamoto, K.R. and Tjian, R. (1985) Cell 42, 559–572.
[13] Graves, B.J., Johnson, P.F. and McKnight, S.L. (1986) Cell 44, 565–576.
[14] Dorn, A., Bollekens, J., Staub, A., Benoist, C. and Mathis, D. (1987) Cell 50, 863–872.
[15] Fletcher, C., Heintz, N. and Roeder, R.G. (1987) Cell 51, 773–781.
[16] Scheidereit, C., Heguy, A. and Roeder, R.G. (1987) Cell 51, 783–793.
[17] Yamamoto, K.R. (1985) Annu. Rev. Genet. 19, 209–252.
[18] Carthew, R.W., Chodosh, L.A. and Sharp, P.A. (1985) Cell 43, 439–448.
[19] Hurst, H.C. and Jonse, N.C. (1987) Genes Dev. 1, 1132–1146.