Short Communication Plant Biotechnology, **17** (4), 297–303 (2000)

Hex-Motif-Specific Binding Protein HBP-1b(c38) can Activate Transcription Without Interacting with a Target DNA Sequence

Takuya ITO,[1,] * Takuya NAKAYAMA,[2] Tetsuo MESHI and Masaki IWABUCHI[3]

Department of Botany, Division of Biological Science, Graduate School of Science, Kyoto University, Sakyo-ku, Kyoto 606-8224, Japan

[1] *Present address: Laboratory of Plant Molecular Biology, The Institute of Physical and Chemical Research (RIKEN), Tsukuba Life Science Center, 3-1-1 Koyadai, Tsukuba, Ibaraki 305-0074, Japan*

[2] *Present address: Department of Cell & Developmental Biology, L215, School of Medicine, Oregon Health Sciences University, 3181 S.W. Sam Jackson Park Road, Portland, Oregon 97201-3098, USA*

[3] *Present address: Research Institute for Biological Science, Okayama, 7549-1 Kayo-cho, Okayama 716-1241, Japan*

**Corresponding author* E-mail address: taito@rtc.riken.go.jp

Received 13 March 2000; accepted 29 June 2000

Abstract

The type I element (CCACGTCACCAATCCGCG) is a *cis*-acting element essential for cell cycle-dependent expression of the wheat histone *H3* gene (TH012). This element consists of two distinct motifs, Hex (CCACGTCA) and Oct (CGCGGATT). To understand the mechanism of transcriptional regulation *via* the type I element, we analyzed the function of HBP-1b(c38), a Hex-motif-specific DNA-binding protein of wheat, by using a transient expression system. Cotransfection experiments with effector and reporter plasmids indicated that overexpressed HBP-1b(c38) did not activate expression of the reporter gene with a normal type I element but unexpectedly enhanced the activity of the promoter with a mutated Hex-motif-containing type I element. This activation was Oct-motif-dependent. Basic, leucine zipper, and acidic regions of the HBP-1b(c38) protein were necessary for this activation. These results suggest that HBP-1b(c38) has an ability to activate transcription without interacting with a target DNA sequence.

Abbreviations

bZIP, basic/leucine zipper; CaMV, cauliflower mosaic virus; CAT, chloramphenicol acetyl transferase; EMSA, electrophoretic mobility shift assay

Introduction

Transcription of eukaryotic class II genes mediated by RNA polymerase II is controlled by several *cis*-acting elements in the regulatory region that are responsive to numerous environmental and physiological signals. These *cis*-acting elements provide binding sites for sequence-specific transcription factors, termed *trans*-acting factors. These factors play important roles in transcriptional regulation (Johnson and McKnight, 1989).

The promoter of the wheat histone *H3* gene (TH012; Tabata *et al.*, 1984) has a type I element (consensus: CCACGTCANCGATCCGCG). This element is conserved among several plant histone genes (Mikami *et al.*, 1994) and is composed of two distinct motifs, Hex (CCACGTCA) and Oct (CGCGGATC). The Hex motif contains the ACGT core sequence and exists in *cis*-regulatory regions of various plant cellular and viral genes and in T-DNA of *Agrobacterium* (Tabata *et al.*, 1991). On the other hand, the Oct motif has been shown to be a *cis*-element specific to plant histone genes (Chaubet *et al.*, 1986; Nakayama *et al.*, 1992). The type I element has been shown to be involved in S-phase-specific regulation of the *H3* gene by loss- and gain-of-function analyses (Ohtsubo *et al.*, 1997; Kaya *et al.*, unpublished). Previously we identified five kinds of DNA-binding proteins that can specifically interact with the Hex motif and showed that these proteins contain bZIP domains and are constituted of two subfamilies, HBP-1a and HBP-1b, based on their binding properties and structural characteristics (Tabata *et al.*, 1989; Tabata *et al.*, 1991; Mikami *et al.*, 1994). The members of the HBP-1a subfamily exhibit a strong binding affinity to the G-box motif (CACGTG) and the Hex motif and a lower affinity to the *as-1* element in the

CaMV 35S promoter (Izawa *et al.*, 1993; Mikami *et al.*, 1994). On the other hand, the members of the HBP-1b subfamily exhibit a strong binding affinity not only to the Hex motif but also to the *as-1* element, and a lower affinity to the G-box motif (Tabata *et al.*, 1991; Mikami *et al.*, 1994). Many plant nuclear proteins bearing bZIP domains have been reported as candidates for transcription factors (Foster *et al.*, 1994; Meshi and Iwabuchi, 1995), and some of the bZIP-type proteins, such as TGAla, TAF-1, Opaque-2, GBF1, and HBP-1a(17), have been shown to function as transcriptional activators (Katagiri *et al.*, 1990; Yamazaki *et al.*, 1990; Oeda *et al.*, 1991; Schindler *et al.*, 1992; Schmidt *et al.*, 1992; Neuhaus *et al.*, 1994; Nakayama *et al.*, 1997). Thus, cDNA clones encoding the HBP-1 family members we had isolated have been supposed to be candidates for transcription factors involved in the regulation *via* the Hex motif of the wheat histone *H3* gene.

To understand the mechanism of transcriptional regulation *via* the Hex motif of the *H3* gene, it is necessary to elucidate the function of individual members of the HBP-1 family. Here, we focused on HBP-1b(c38), one of the HBP-1b subfamily members, because the *1b(c38)* gene is coordinately expressed with the *H3* gene during germination and has been suggested to be involved in the transcriptional regulation of the *H3* gene (Minami *et al.*, 1993). As the type I element exists commonly in both monocots and dicots (Mikami *et al.*, 1994), this suggests that transcription *via* the type I element is common to plant species. Therefore, in this study, we adopted a transient expression assay using heterologous tobacco mesophyll protoplasts because the tobacco system is well established. We show that 1b(c38) does not transactivate a promoter with a normal type I element but can transactivate a promoter with a Hex-motif-mutated type I element. This activation is Oct-motif-dependent. These results suggest that HBP-1b(c38) has an ability to activate transcription without interacting with a target DNA sequence.

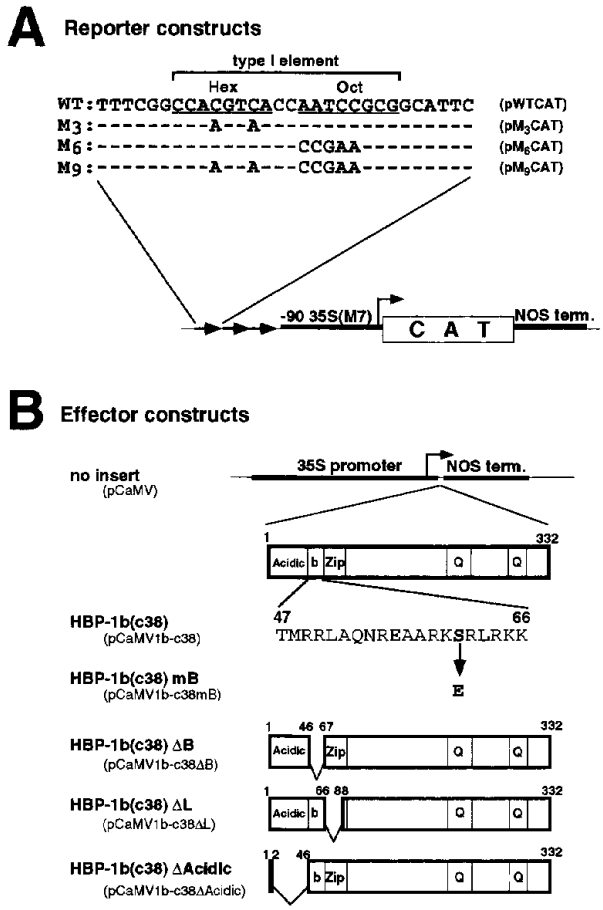

Fig. 1 Schematic representation of reporter and effector constructs for cotransfection assay. (A) Reporter constructs consisting of three copies of wild-type or mutated type I element, truncated CaMV 35S promoter (-90 35S [M7]), and the coding region for *CAT*. Hex and Oct motifs are underlined, and the type I element is bracketed. In each mutated type I element (M_3, M_6, and M_9), only substituted bases are indicated. (B) Effector constructs consisting of the CaMV 35S promoter and cDNA for HBP-1b(c38) derivatives. The amino acid sequence of the basic region is shown in the HBP-1b(c38) row, and a substituted amino acid residue is shown in the HBP-1b(c38)mB row. Acidic, b, Zip, and Q represent acidic, basic, leucine zipper, and glutamine-rich regions, respectively.

Materials and Methods

Plasmid construction

A CAT expression reporter plasmid, pWTCAT, was driven by the CaMV 35S (-90) promoter with three repeated sequences of the H3 type I element (**Fig. 1A**). Three types of mutant reporter plasmids, pM_3CAT, pM_6CAT, and pM_9CAT, were constructed, in which Hex, Oct, and both motifs were changed, respectively.

To prepare a series of effector plasmids, we initially constructed a plasmid, pCaMV, by ligating a *Bam*HI-*Sac*I backbone of pBI221 (Clontech Laboratories, Inc., Palo Alto, CA, USA). A wild-type effector plasmid, pCaMV1b-c38, was made as follows. A 2.2-kb *Eco*RI fragment of 1b-c38 in M13mp19 (Tabata *et al.*, 1991) was used as a starting cDNA for HBP-1b(c38). By using oligonucleotide mutagenesis, bases at positions between -9 and -4 (the A residue of the ATG start codon of HBP-1b(c38) was +1) were changed from TAATCT to GGATCC (*Bam*HI site). From this

mutated construct, a *Bam*HI (at-9)-*Hha* I (at +1002) fragment containing an open reading frame of HBP-1b(c38) was isolated. This fragment, the *Bam*HI-*Sac*I backbone of pBI221, and two complimentary oligonucleotides, 5'-TCGACGCATGCA-GCT-3', and 5'-GCATGCGTCGACG-3' were ligated to give pCaMVlb-c38. Effector plasmids for HBP-1b(c38) derivatives were also prepared (**Fig. 1B**).

Preparation of protoplasts and transformation

Protoplasts from tobacco (*Nicotiana tabacum* cv. Petit Havana, SR1) mesophyll cells were prepared as described by Lepetit *et al.* (1992). To introduce the reporter and effector plasmids into protoplasts, electroporation was done as described by Ito *et al.* (1995). After electroporation, the protoplasts were kept on ice for 10 min, washed once with the protoplast culture medium, then incubated in the same medium at 26 ℃ in dark.

CAT assay

Electroporated protoplasts were cultured for 24 h, harvested, and then sonicated in 0.2 m*l* of 0.25 M Tris-HCl (pH 7.6), followed by incubation at 65 ℃ for 5 min. Lysates were centrifuged at 15 000 rpm for 5 min at 4℃, and supernatants containing the same amount of proteins were assayed for CAT by using a FluoReporter FAST CAT Gene Detection Kit (Molecular Probes, Inc., Eugene, OR, USA).

Preparation of bacterially expressed recombinant proteins

The coding fragments of HBP-1b(c38) were subcloned into the blunt-ended *Bam*HI site of pET15b (Novagen), and the constructs were expressed in *Escherichia coli* strain BL21(DE3)pLysS according to the manufacturer's instruction. IPTG-induced cells were lysed by sonication in binding buffer (20 mM Tris-HCl [pH 7.9] at 4 ℃, 500 mM NaCl, 1 mM phenylmethylsulfonyl fluoride, and 10% [v/v] glycerol) containing 5 mM imidazole, and each soluble fraction was loaded onto an affinity column (HiTrap Chelating 1 m*l*; Pharmacia Biotech, Uppsala, Sweden) equilibrated with Ni^{2+}. Recombinant proteins were eluted with binding buffer containing 300 mM imidazole. Eluted His-tagged HBP-1b(c38) proteins were used for EMSA. His-tagged HBP-1b(c38)ΔAcidic protein could not be obtained; it probably formed insoluble aggregates in the bacterial cells.

EMSA

Double-stranded synthetic 30-mer DNA was 5'-end-labeled with T4 polynucleotide kinase and [γ-^{32}P]-ATP, and then used as a probe. The nucleotide sequences of the competitors (W.T., HexM, and OctM in **Fig. 3A**) correspond to those of the type I elements of the reporter constructs in **Fig. 1**. The binding reaction mixture containing bacterially expressed recombinant protein, 1 ng probe, 2.5 μg poly d(I-C), competitor DNA, 20 mM HEPES (pH 7.9), 100 mM KCl, 10 mM EDTA, 1 mM dithiothreitol, 2.4% Ficoll, and 0.012% Nonidet P-40, was incubated for 30 min at room temperature in a final volume of 14 μl and then loaded on a 5% native polyacrylamide gel containing 0.15X Tris-borate EDTA buffer at 4 ℃.

Immunoblot assay

To detect expression of HBP-1b(c38) protein and its derivatives in plant cells, cell extracts prepared from transfected mesophyll protoplasts (1×10^5 cells mL^{-1}) were fractionated by SDS-PAGE and assayed by immunoblotting with anti-His-tagged HBP-1b(c38) rabbit antiserum using an Amplified Alkaline Phosphatase Immun-Blot Assay Kit (Bio-Rad Laboratories Inc., Richmond, CA, USA).

Results and Discussion

To determine the function of HBP-1b(c38) *via* the type I element, we examined whether HBP-1b(c38) can transactivate or transrepress the expression of reporter genes with the type I element. As shown in **Fig. 1A**, the reporter genes possess the coding region of the CAT gene, driven by a truncated CaMV 35S promoter (up to -90 bp). Because the 35S promoter originally had two sets of the TGACG motif in the *as-1* element, to which HBP-1b(c38) can bind *in vitro*, both sets were changed to TTACTT not to bind to HBP-1b(c38) (Kawata *et al.*, unpublished). Three copies of the type I element were joined in tandem to the upstream region of the truncated promoter.

When the wild-type reporter plasmid (WT; **Fig. 1A**) was introduced into tobacco mesophyll protoplasts together with an effector plasmid expressing HBP-1b(c38) (**Fig. 1B**), no significant change in CAT activity was observed (**Fig. 2A**, columns 1 and 2). But, unexpectedly, the expression of the M_3 reporter gene (**Fig. 1A**) with a mutated Hex motif was apparently activated (**Fig. 2A**, columns 3 and 4). When M_6 and M_9 reporter plasmids (**Fig. 1A**) were used, activation by HBP-1b(c38) was not observed (**Fig. 2A**, columns 5—8), which indicates that the stimulatory effect of HBP-1b(c38) on the M_3 reporter is Oct-motif-dependent. To examine whether the effector plasmid really expresses HBP-1b(c38) protein in transfected protoplasts, protoplast

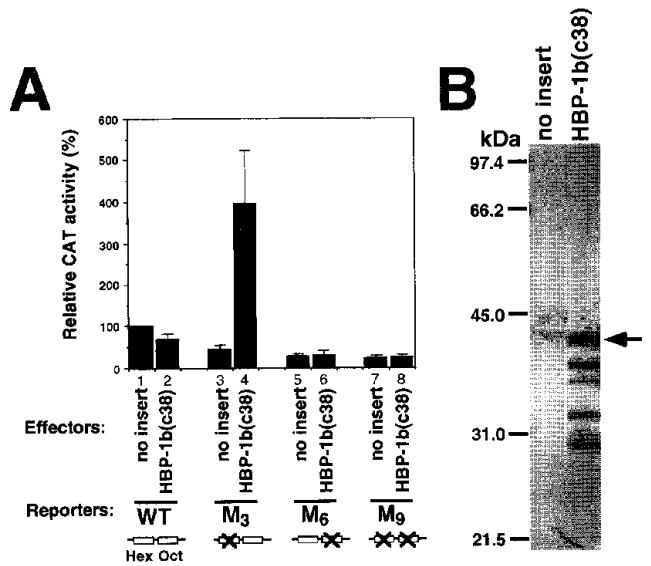

Fig. 2 Transactivation of type I element-containing reporter genes by HBP-1b(c38) in tobacco mesophyll protoplasts. (A) Cotransfection experiments. Fifteen micrograms of the reporter plasmid, 40 μg of the effector plasmid, and 100 μg of sonicated calf thymus DNA were added to 0.5 m*l* of protoplast suspension. Relative CAT activity (%) was calculated on the basis of the activity of the control (100%; column 1) (B) Immunoblot analysis of the extract from tobacco mesophyll protoplasts transfected with an effector plasmid. Quantities as in panel A. The arrow indicates the band corresponding to HBP-1b(c38). Bands with lower molecular weights probably indicate degraded products of HBP-1b(c38).

lysates were prepared and then assayed by immunoblotting using antiserum against bacterially expressed HBP-1b(c38). The results showed that HBP-1b(c38) was synthesized in transfected protoplasts (**Fig. 2B**). We also showed that binding of HBP-1b(c38) to the Hex motif was prevented by the mutation in the Hex motif but was not influenced by altering the sequence of the Oct motif by using competitive EMSA (**Fig. 3A**). We also confirmed that HBP-1b(c38) could not bind to the M_3 probe (data not shown). From these results, it was assumed that HBP-1b(c38) can work as a positive factor through interaction with Oct-containing motif, only when it cannot interact with the Hex motif.

Next, we prepared four kinds of HBP-1b(c38) derivatives, mB, ΔB, ΔL, and ΔAcidic (**Fig. 1B**) to identify functional domains of the HBP-1b(c38) protein necessary for M_3 reporter activation and examined their transactivation abilities. HBP-1b(c38)mB is a derivative in which a serine residue in the basic region is changed to a glutamic acid residue. This serine residue is conserved in almost all the plant bZIP-type proteins reported to date (data not shown). HBP-1b(c38)ΔB, ΔL, and ΔAcidic lack the basic, leucine zipper, and acidic regions, respectively. As shown in **Fig. 4A**, mB activated transcription and the other derivatives did not (columns 3—6). Immunoblot analysis showed that HBP-1b(c38) and its derivatives were expressed at a comparable level in tobacco mesophyll protoplasts (**Fig. 4B**). We then examined DNA-binding activity of HBP-1b(c38) derivatives by EMSA with partially purified His-tagged fusion proteins (**Fig. 3B** and **3C**). **Fig. 3C** shows that neither HBP-1b(c38)mB, ΔB nor ΔL could bind to the type I element. Similarly, these mutated proteins did not bind to the Hex-mutated type I element (data not shown). These results indicate that acidic, basic, and leucine zipper regions are necessary for M_3 reporter activation and that DNA-binding ability of HBP-1b(c38) is not necessary for this activation.

In this paper, we showed that HBP-1b(c38) could not activate the WT reporter gene, but activated reporter gene expression Oct-motif-dependently only when Hex motif was mutated (**Fig. 2**). The above seemingly contradictory results can be interpreted by either of the two possible explanations. One of them is that HBP-1b(c38) negatively regulates transcription Hex-motif-dependently. Recently, both activators and repressors were found among *Arabidopsis* ethylene-responsive element binding factors (Fujimoto *et al.*, 2000). In the case of HBP-1 family, activators and repressors may cooperatively regulate transcription of the histone genes. The other explanation of the M_3 reporter activation is that HBP-1b(c38) activated transcription through direct or indirect interaction with Oct-binding factor of tobacco. We have no direct evidence of interaction between HBP-1b(c38) and Oct-binding factor. However, the protein—protein interactions of DNA-binding transcription factors have been reported in many cases; for example, between c-Jun and glucocorticoid receptor or Myo-D (Yang-Yen *et al.*, 1990; Schüle *et al.*, 1990; Bengal *et al.*, 1992), and between C/EBP and NF-κB (Stein *et al.*, 1993). In these cases, one functions to the other without binding to its target sequence. We have indicated that acidic, basic and leucine zipper regions were necessary for M_3 reporter activation (ΔB, ΔL and ΔAcidic in **Fig. 4A**). These results support that there may be the protein—protein interaction between HBP-1b(c38) and presumed tobacco factor.

It has to be elucidated what M_3 gene activation by HBP-1b(c38) means *in vivo*. We think that it

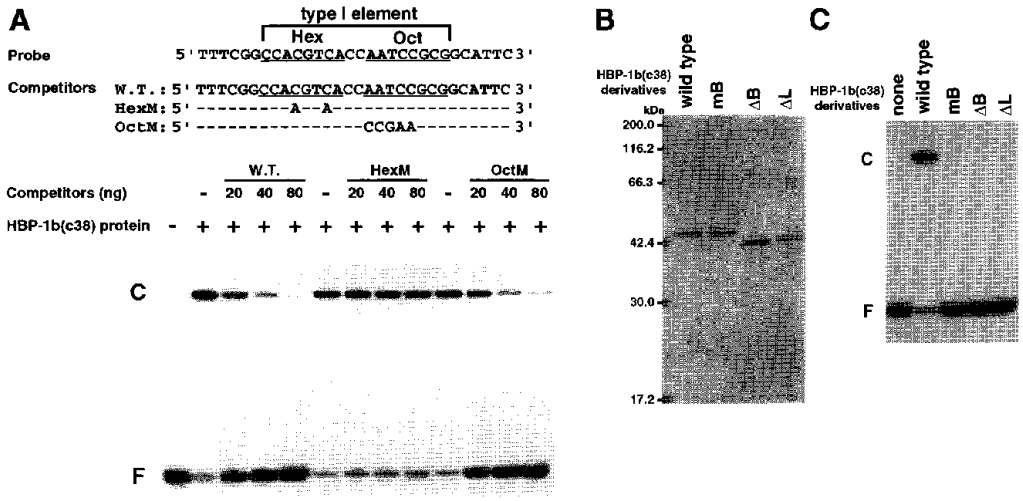

Fig. 3 DNA binding of HBP-1b(c38) and its derivatives to the type I element *in vitro*. (A) Competitive mobility shift assay of HBP-1b(c38). His-tagged HBP-1b(c38) protein was expressed in *E. coli* and partially purified by Ni^{2+} affinity column chromatography, as described in Materials and Methods. Double-stranded 30-mer oligonucleotides used as probes and competitors were synthesized. In the three competitors, only substituted bases are indicated. Hex and Oct motifs are underlined, and the type I element is bracketed. F, free probe; C, DNA-protein complex. (B) SDS-PAGE analysis. Partially purified His-tagged HBP-1b(c38) derivatives were loaded on a 12% SDS-polyacrylamide gel and stained with Coomassie Brilliant Blue. Molecular weight markers are shown on the left. (C) EMSA experiment. One-fifth of the amount of proteins used in panel B was incubated with the same probe as used in panel A and subjected to EMSA. F, free probe; C, DNA—protein complex.

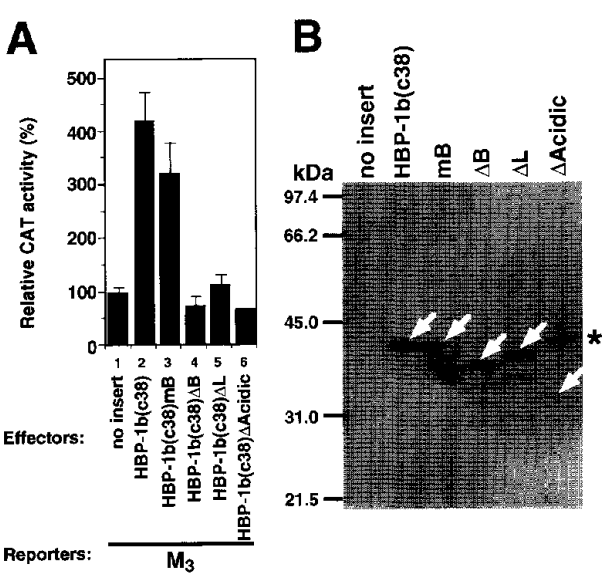

Fig. 4 Transactivation of the M_3 reporter gene by HBP-1b(c38) and its derivatives. (A) Cotransfection experiments. Relative CAT activities were calculated as for Fig. 2. Values are averages of three separate experiments; bars indicate standard errors. (B) Immunoblot analysis of the extract from tobacco mesophyll protoplasts transfected with HBP-1b(c38) or its mutants. Quantities as in Fig. 2. Arrows indicate bands corresponding to HBP-1b(c38) derivatives. A band shown by asterisk was not reproducible, so it may be an artifact.

reflects the transcriptional function of HBP-1b(c38) from the wheat histone promoter. In plant histone gene promoters, Taoka *et al.* (1999) showed that at least three kinds of Oct-motif-containing *cis*-elements—types I, II and III elements—exist. Type I consists of Hex and Oct motifs, type II of TCA and Oct motifs, and type III of CCAAT-box and Oct motifs. Activation in this study might reflect the function of HBP-1b(c38) *via* type II, III or other unknown *cis*-elements lacking the Hex motif but containing the Oct motif. We have to examine these possibilities by introducing mutations into Hex motif other than mutation in the M_3 reporter gene.

In this study, we examined the function of wheat HBP-1b(c38) in a heterologous expression system using tobacco cells. At first we had tried to use a homologous system with cultured wheat cells. However, in this system, no reliable data were obtained because the expression efficiency of all the reporter genes was extreamly low. Thus, further studies are needed to elucidate the real function of HBP-1b(c38) *in vivo* in the homologous system such as a transgenic wheat system.

Acknowledgments

We thank other members of our laboratory for helpful discussions. We also thank M. Okanami for providing pET1b-c38 and anti-HBP-1b(c38) rabbit antiserum. This work was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan to M.I. and T.M.

References

Bengal, E., Ransone, L., Scharfmann, R., Dwarki, V.J., Tapscott, S.J., Weintraub, H. and Verma, I.M., 1992. Functional antagonism between c-Jun and MyoD proteins: a direct physical association. Cell, **68:** 507-519.

Chaubet, N., Philipps, G., Chabout,e M.E., Ehling, M. and Gigot, C., 1986. Nucleotide sequences of two corn histone H3 genes. Genomic organization of the corn histone H3 and H4 genes. Plant Mol. Biol., **6:** 253-263.

Foster, R., Izawa, T. and Chua, N.-H., 1994. Plant bZIP proteins gather at ACGT elements. FASEB J., **8:** 192-200.

Fujimoto, S.Y., Ohta, M., Usui, A., Shinshi, H. and Ohme-Takagi, M., 2000. Arabidopsis ethylene-responsive element binding factors act as transcriptional activators or repressors of GCC box-mediated gene expression. Plant Cell, **12:** 393-404.

Ito, T., Fujimoto, Y., Nakayama, T. and Iwabuchi, M., 1995. A far-upstream sequence of the wheat histone H3 promoter functions differently in rice and tobacco cultured cells. Plant Cell Physiol., **36:** 1281-1289.

Izawa, T., Foster, R. and Chua, N.-H., 1993. Plant bZIP protein DNA binding specificity. J. Mol. Biol., **230:** 1131-1144.

Johnson, R.F. and McKnight, S.L., 1989. Eukaryotic transcriptional regulatory proteins. Annu. Rev. Biochem., **58:** 799-839.

Katagiri, F., Yamazaki, K., Horikoshi, M., Roeder, R.G. and Chua, N.-H., 1990. A plant DNA-binding protein increases the number of active preinitiation complexes in a human in vitro transcription system. Genes Dev., **4:** 1899-1909.

Lepetit, M., Ehling, M., Chaubet, N. and Gigot, C., 1992. A plant histone gene promoter can direct both replication-dependent and -independent gene expression in transgenic plants. Mol. Gen. Genet., **231:** 276-285.

Meshi, T. and Iwabuchi, M., 1995. Plant transcription factors. Plant Cell Physiol., **36:** 1405-1420.

Mikami, K., Sakamoto, A. and Iwabuchi, M., 1994. The HBP-1 family of wheat basic/leucine zipper proteins interacts with overlapping *cis*-acting hexamer motifs of plant histone genes. J. Biol. Chem., **269:** 9974-9985.

Minami, M., Huh, G.H., Yang, P. and Iwabuchi, M., 1993. Coordinate gene expression of five subclass histones and the putative transcription factors, HBP-1a and HBP-1b, of histone genes in wheat. Plant Mol. Biol., **23:** 429-434.

Nakayama, T., Okanami, M., Meshi, T. and Iwabuchi, M., 1997. Dissection of the wheat transcription factor HBP-1a(17) reveals a modular structure for the activation domain. Mol. Gen. Genet., **253:** 553-561.

Nakayama, T., Sakamoto, A., Yang, P., Minami, M., Fujimoto, Y., Ito, T. and Iwabuchi, M., 1992. Highly conserved hexamer, Octamer and nonamer motifs are positive *cis*-regulatory elements of the wheat histone H3 gene. FEBS Lett., **300:** 167-170.

Neuhaus, G., Neuhaus-Url, G., Katagiri, F., Seipel, K. and Chua, N.-H., 1994. Tissue-specific expression of as-1 in transgenic tobacco. Plant Cell, **6:** 827-834.

Oeda, K., Salinas, J., Chua, N.-H., 1991. A tobacco bZip transcription activator (TAF-1) binds to a G-box-like motif conserved in plant genes. EMBO J., **10:** 1793-1802.

Ohtsubo, N., Nakayama, T., Kaya, H., Terada, R., Shimamoto, K., Meshi, T. and Iwabuchi, M., 1997. Cooperation of two distinct *cis*-acting elements is necessary for the S phase-specific activation of the wheat histone H3 promoter. Plant J., **11:** 1219-1225.

Schindler, U., Terzaghi, W., Beckmann, H., Kadesch, T. and Cashmore, A.R., 1992. DNA binding site preferences and transcriptional activation properties of the *Arabidopsis* transcription factor GBF1. EMBO J., **11:** 1275-1289.

Schmidt, R.J., Ketudat, M., Aukerman, M.J. and Hoschek, G., 1992. Opaque-2 is a transcriptional activator that recognizes a specific target site in 22-kD zein genes. Plant Cell, **4:** 689-700.

Schüle, R., Rangarajan, P., Kliewer, S., Ransone, L.J., Bolado, J., Yang, N., Verma, I.M. and Evans, R.M., 1990. Functional antagonism between oncoprotein c-Jun and the glucocorticoid recepor. Cell, **62:** 1217-1226.

Stein, B., Cogswell, P.C., Baldwin Jr., A.S., 1993. Functional and physical associations between NF-κB and C/EBP family members: a Rel domain—bZIP interaction. Mol. Cell. Biol., **13:** 3964-3974.

Tabata, T., Fukasawa, M. and Iwabuchi, M., 1984. Nucleotide sequence and genomic organization of a wheat histone H3 gene. Mol. Gen. Genet., **196:** 397-400.

Tabata, T., Nakayama, T., Mikami, K. and Iwabuchi, M., 1991. HBP-1a and HBP-1b: leucine zipper-type transcription factors of wheat. EMBO J., **10:** 1459-1467.

Tabata, T., Takase, H., Takayama, S., Mikami, K., Nakatsuka, A., Kawata, T., Nakayama, T. and Iwabuchi, M., 1989. A protein that binds to a *cis*-acting element of wheat histone genes has a leucine zipper motif. Science, **245:** 965-967.

Taoka, K.-i., Kaya, H., Nakayama, T., Araki, T., Meshi, T. and Iwabuchi, M., 1999. Identification of three kinds of mutually related composite elements conferring S phase-specific transcriptional activation. Plant J., **18:** 611-623.

Yamazaki, K., Katagiri, F., Imaseki, H. and Chua, N.-H., 1990. TGA1a, a tobacco DNA-binding protein, increases the rate of initiation in a plant in vitro transcrip-

tion system. Proc. Natl. Acad. Sci. USA, **87:** 7035-7039.

Yang-Yen, H.-F., Chambard, J.-C., Sun, Y.-L., Smeal, T., Schmidt, T.J., Drouin, J. and Karin, M., 1990. Transcriptional interference between c-Jun and the glucocorticoid receptor: mutual inhibition of DNA binding due to direct protein—protein interaction. Cell, **62:** 1205-1215.