A cDNA clone encoding HBP-1b homologue in Arabidopsis thaliana

Takefumi Kawata, Takuya Imada, Hideaki Shiraishi, Kiyotaka Okada, Yoshiro Shimura and Masaki Iwabuchi

Division of Developmental Biology, 2Division of Gene Expression and Regulation I, National Institute for Basic Biology, Okazaki 444, 3Department of Botany and 4Department of Biophysics, Faculty of Science, Kyoto University, Kyoto 606-01, Japan

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Our researches on the transcriptional regulation of wheat histone H3 gene have revealed the presence of several cis- and trans-acting factors (1). One of the best characterized regulatory sequences is the hexameric motif, ACATCA (2), and we have identified two distinct hexamer-specific DNA-binding proteins, HBP-1a and HBP-1b, in wheat (3). HBP-1b can bind to the hexameric motifs found in the CaMV35S and NOS promoters as well as the hexameric motif of the H3 promoter, whereas the HBP-1a binds exclusively to the H3 promoter (3, 4). We have already isolated cDNA clones encoding HBP-1a and HBP-1b from wheat (5, 6). Structural analyses of the cDNAs revealed that both proteins are member of the bZIP-type transcription factors (7). HBP-1b-like factors capable of binding to the hexameric (or related) motif of several T-DNA and plant viral gene promoters have been found as OCSTF in maize (8) and as ASF-1 in tobacco (9), and their cognate cDNAs, OCSBF-1, -2 (10), and TGA1a (11), have been isolated. All of these have bZIP domains and similar DNA-binding specificity. This prompted us to speculate the genes of HBP-1b homologues may widely be present in various plant species. Isolation of the HBP-1b homologues from various plants is necessary to clarify this issue. Furthermore, function of HBP-1b is not known yet. To understand the function of HBP-1b, it will be important to study the transcription factor by genetic approaches as well as biochemical ones. In this respect, we isolated a cDNA clone for HBP-1b counterpart from Arabidopsis thaliana which is a useful plant for genetic analyses.

We firstly screened a genomic library of A. thaliana (cv. Landsberg) and obtained one positive clone out of $1 \times 10^8$ plaques by using wheat HBP-1b cDNA as a probe. The genomic clone was used as a probe to isolate a cDNA clone from a cDNA library in λZAPII which had been prepared with poly(A)+RNA from 20-days-old A. thaliana (cv. Landsberg) aerial part tissues. Under high stringent conditions (1×SSC, 60°C), five overlapping cDNA clones were obtained, and a cDNA clone (bA19) containing the longest insert was sequenced. DNA sequence analysis revealed that the bA19 clone had a 1654 bp insert that contains a single open reading frame (ORF) beginning at position 267. The ORF encodes a polypeptide consisting of 330 amino acids with calculated molecular weight of 36,684. A deduced amino acid sequence contains a bZIP domain in the N-terminal portion of the protein with 100% homology to the wheat HBP-1b (Fig. 1). Therefore, we conclude that the bA19 clone contains the DNA sequences encoding a HBP-1b counterpart in A. thaliana. A homology of the overall amino acid sequences between the bA19-encoded protein and wheat HBP-1b (c-38) is 75%, whereas the bA19 protein shared 53% and 33% homology with TGA1a and OCSBF-1, respectively. Isolation of the HBP-1b counterpart from Arabidopsis would confer understanding of the real HBP-1b functions in vivo by using the T-DNA tagging and gene disruption techniques. During the preparation of this manuscript, a cDNA clone (PosF21) encoding a bZIP-type DNA binding protein has been reported in A. thaliana (cv. Columbia) (12). There is no obvious homology in amino acid sequence between bA19 and PosF21, thus they are different transcription factors in A. thaliana.

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REFERENCES