Journal of Experimental Botany, Vol. 53, No. 373, pp. 1531–1532, June 2002

Journal of Experimental Botany

GENE NOTE

Cloning of a tobacco cDNA coding for a putative transcriptional coactivator MBF1 that interacts with the tomato mosaic virus movement protein

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Received 3 January 2002; Accepted 4 March 2002

Abstract

Viral movement through plasmodesmata in host plants depends on the interaction between virus-encoded movement protein (MP) and host proteins. To search for MP-interacting protein (MIP), far-western screening of a tobacco cDNA library was carried out using a recombinant MP of tomato mosaic virus (ToMV) as a probe. One of the positive cDNA clones, designated MIP204, was highly homologous to a class of transcriptional coactivators commonly referred to as multiprotein bridging factor 1 (MBF1). ToMV MP could also bind to the *Arabidopsis* homologues of MBF1. The recombinant MIP204 bound to MPs of ToMV and a crucifer tobamovirus CTMV-W, but not of cucumber mosaic virus. MPs of ToMV and the related virus may interact with MBF1-like proteins to modulate host gene expression.

Key words: Movement protein, multiprotein bridging factor 1, tomato mosaic virus, transcriptional coactivator.

Plant viruses utilize their own gene product called movement protein (MP) to enable their cell-to-cell and long-distance movement through plasmodesmata and the vascular system (Carrington *et al*., 1996). MP binds to the viral genome and interacts with various host factors (Dorokhov *et al*., 1999; Ham *et al*., 1999; Lazarowitz and Beachy, 1999; Chen *et al*., 2000; Soellick *et al*., 2000; Huang *et al*., 2001; Lin and Heaton, 2001; Matsushita *et al*., 2001). Recent reports suggest that regulatory roles of MP may extend for additional events such as modulation of host gene expression (Voinnet *et al*., 2000; Aranda and Maule, 1998). In this regard, it was recently reported that a putative transcriptional coactivator KELP (Cormack *et al*., 1998) interacts with MPs of tomato mosaic virus (ToMV), a crucifer tobamovirus CTMV-W and cucumber mosaic virus (CMV) (Matsushita *et al*., 2001). In the present study, a cDNA coding for another MP-interacting protein (MIP), which is also a transcritional coactivator and was first reported as multiprotein bridging factor 1 (MBF1) of the silkworm *Bombyx mori* Takemaru *et al*., 1997), has been cloned and characterized.

In order to identify tobacco proteins that interact with ToMV MP, a cDNA library (Matsushita *et al*., 2001) constructed with a phage expression vector λGEX5 and mRNA of *Nicotiana tabacum* cv. Samsun NN was screened. Glutathione *S*-transferase (GST)-fused ToMV MP (GST-PKA-MP) was phosphorylated with [γ-^{32}P]ATP and used as a probe for far-western screening of the cDNA products immobilized on nitrocellulose membrane (Matsushita *et al*., 2001). Sequencing analysis of positive clones resulted in the identification of a clone, MIP204, whose amino acid sequence was 40–92% homologous (data not shown) to MBF1 from various organisms including plants (Goday *et al*., 2001; GenBank: AV442382 and AL353032), human (GenBank: AB002282), silkworm (Takemaru *et al*., 1997), and yeast (GenBank: AB017593). The nucleotide sequence of MIP204 has been submitted to DDBJ (AB072698).

Southern blot analyses of the *MIP204* gene indicated that there may be multiple related genes in *N. tabacum* (data not shown). Thus, the gene encoding MIP204 was named as *NtMBF1a*. Occurrences of multiple MBF1 genes are known in other organisms including *A. thaliana* (GenBank: AV442382 and AL353032). Based on the N-terminal homology with other MBF1 proteins from various plants, the first ATG codon of MIP204 was assumed to be the translational initiation codon, which was followed by a full-length open reading frame (ORF).

It was tested whether the *Arabidopsis* MBF1-like proteins could also bind to ToMV MP. The two *Arabidopsis* cDNA, which are referred to as AtMBF1a (GenBank: AV442382) and AtMBF1b (GenBank: AL353032, protein 10: CAB88285.1), were amplified from a cDNA library of *A. thaliana* Col (Matsushita *et al*., 2001) by PCR using the primer sets AtNt4F01 (5′-ACGAATTCATGGCCGGAATTGGACCG-3′) and AtNt4R01 (5′-AACTCGAGACGAAAGCATTGATTCTTGT-3′), and AtNt4F02 (5′-ACGAATTCATGGCCGGAATTGGACCGATT-3′) and AtNt4R02 (5′-AACTCGAGCTACTTCTTTCCACGGAGT-3′), respectively. The amplified cDNAs were inserted into pGEX-6P-1 (Amersham Pharmacia Biotech) to construct pGEX-P-At30KNt4A and pGEX-P-At30KNt4B for the expression of GST-AtMBF1a and GST-AtMBF1b, respectively. As a positive control, *NtMBF1a* ORF was also inserted into the same vector to construct pGEX-P-d5U30KNt4 to express GST-NtMBF1a. These recombinant proteins were subjected to protein-binding assays with ToMV MP as a probe. As shown in Fig. 1, both GST-AtMBF1a and GST-AtMBF1b showed MP-binding activity as well as GST-NtMBF1a, while a negative control GST did not show any binding activity.

It was further tested whether NtMBF1a could bind to MPs of other plant viruses such as CMV and CTMV-W. The recombinant MPs, purified as described previously (Matsushita *et al*., 2001), were immobilized on a nitrocellulose membrane and subjected to a far-western analysis. To prepare the NtMBF1a probe, a plasmid pGEX-P-PKA-d5U30KNt4 was constructed for the production of GST-PKA-NtMBF1a. This recombinant protein purified on Glutathione Sepharose beads was phosphorylated with protein kinase A and subsequently treated with PreScission Protease (Amersham Pharmacia Biotech) to elute the GST-free probe PKA-NtMBF1a. As shown in Fig. 2, ^{32}P-labelled PKA-NtMBF1a bound to MPs of ToMV and CTMV-W, but not of CMV. The failure in CMV MP binding was distinct from the case for the

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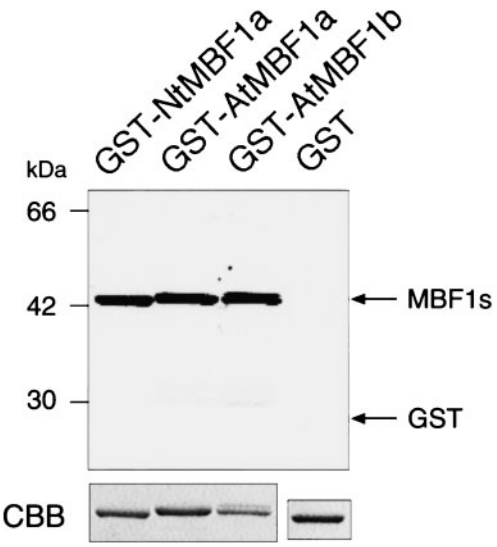

Fig. 1. Binding of ToMV MP to MBF1s of tobacco and *Arabidopsis*. Affinity-purified GST-NtMBF1a, GST-AtMBF1a, GST-AtMBF1b, and GST were resolved in 12.5% SDS-polyacrylamide gel and blotted onto a PVDF membrane. After renaturation of the proteins, the blot was probed with ^{32}P-labelled PKA-MP. The autoradiogram and Coomassie blue staining were shown in the upper and lower panels. Arrows show the positions of the recombinant proteins. Positions of molecular mass (kDa) markers are shown on the left.

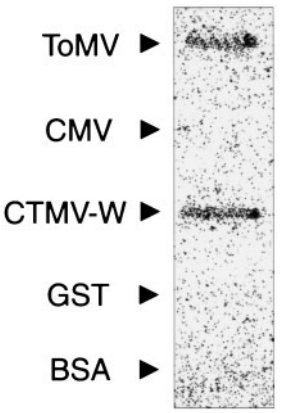

Fig. 2. Binding of NtMBF1a to MPs of various viruses. Equal amount (1 μg) of affinity-purified GST-MP (ToMV), GST-CMVMP (CMV), GST-CTMVMP (CTMV-W), GST, and bovine serum albumin (BSA) were immobilized onto a nitrocellulose membrane using a slot-blotter and probed with ^{32}P-labelled PKA-NtMBF1a. The recombinant proteins GST-CMVMP and GST-CTMVMP were described previously (Matsushita *et al.*, 2001).

previously reported transcriptional coactivator KELP (Matsushita *et al.*, 2001), which was shown to bind to CMV MP.

Transcriptional coactivators are a new class of transcription factors, which play a crucial role in gene expression by interconnecting a regulator DNA-binding protein with a component of the basal transcription machinery such as TATA-binding protein (Cormack *et al.*, 1998; Takemaru *et al.*, 1997). In plants, one of the tomato MBF1-like proteins ER24 was up-regulated in fruit by ethylene treatment and during ripening (Zegzouti *et al.*, 1999). It was also reported that potato StMBF1 was up-regulated in tubers by fungal infection, wounding, and treatment of chemicals such as salicylic acid and an ethylene precursor, ethephon (Goday *et al.*, 2001). These suggest the possible roles of plant MBF1 during normal physiological processes and the general defence response against pathogens. The finding that tobamovirus MP bound to plant MBF1 raised the intriguing possibility that MPs of plant viruses could modulate host gene expression to suppress the general defence response in plants.

Acknowledgements

This work was supported in part by the Grant-in-Aid for Encouragement of Young Scientists and one for Scientific Research on Priority Areas from the Ministry of Education, Culture, Sports, Science, and Technology of Japan. It was also supported by the Grant-in-Aid 'Integrated Research Program for the Development of Innovative Plants and Animals using Transformation and Cloning' from the Ministry of Agriculture, Forestry and Fisheries of Japan.

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