Short Communication

The CaTin1 (Capsicum annuum TMV-induced Clone 1) and CaTin1-2 Genes are Linked Head-to-Head and Share a Bidirectional Promoter

Ryoung Shin 1, Min Jung Kim and Kyung-Hee Paek 2

Graduate School of Biotechnology, Korea University, 1,5ga Anam-dong, Sungbuk-gu, Seoul 136-701, Republic of Korea

CaTin1 was expressed relatively early in the TMV-inoculated leaves of hot pepper which is resistant to TMV-P0 infection. Interestingly, there was another homologous gene (CaTin1-2) located in front of CaTin1 in a head-to-head fashion and they shared a single promoter. The expression profile of the CaTin1-2 was very similar to CaTin1 in all the treatments except the slower induction time compared to CaTin1 upon TMV-P0 inoculation. The promoter analysis of CaTin1 and CaTin1-2 revealed bidirectionality both in cis-elements and activity. The CaTin1-2 promoter had two TATA-boxes, four GCC-boxes, the root responsive element, and a W1-box. The ethylene-inducible promoter activity depended on GCC-boxes and TMV-inducible activity of the CaTin1-2 promoter reached its highest activity when this promoter had a W1-box.

Keywords: Bidirectional promoter — Ethylene — GCC-box — Hot pepper — TMV — W1-box.

Abbreviations: MeJA, methyl jasmonic acid; MV, methyl violoegen; PR, pathogenesis-related; SA, salicylic acid; SAR, systemic acquired resistance; TMV, tobacco mosaic virus.

The nucleotide sequence reported in this paper for CaTin1-2 has been submitted to EMBL GenBank under accession number AF480414.

Most eukaryotic genes are separated by thousands of nucleotides and are transcribed by unidirectional promoters. However, there are several examples of closely spaced and divergently transcribed mammalian genes under the influence of a common bidirectional promoter (Hentchel and Birnstiel 1981, Huang et al. 1990, Killen et al. 1988, Saffer and Singer 1984). Bidirectional promoters have also been found in several genes expressed at high level in specific tissues in mammalian cells (Pollner et al. 1990). In plants, since Shwarz et al. (1981) reported the first example of bidirectional promoter of chloroplast genes from maize, several chloroplast genes with bidirectional promoter have been reported (Berends et al. 1987, Kolchi et al. 1988, Meng et al. 1991). In the case of nuclear genes, a bidirectional promoter between a seed-specific oleosin gene and a putative methionine sulphotide reductase gene has been reported (Keddie et al. 1994, Sadanandom et al. 1996). In plants, differently from animals, so far there is no other report of a bidirectional promoter.

In a previous study, we isolated N6-11 gene that was one of the genes induced specifically during incompatible interaction of hot pepper with TMV (Shin et al. 2001). This clone, renamed Capsicum annuum TMV-induced clone 1 (CaTin1), contained an insert of 775 bp encoding a protein of 180 amino acid residues. Using the CaTin1 gene-specific primer 1 (5’-AGCGTGAAATGAGAAGACCGAGATGGAGATGAGA-3’) and 2 (5’-GGAACAGAGAATTGGTTACTCATGGCTACCTGAC-3’), we located and obtained about 1.0 kb region of the CaTin1 promoter from the adaptor-ligated genomic DNA fragments in GenomeWalker libraries, according to the manufacturer’s instructions (CLONTECH, Palo Alto, U.S.A.). Surprisingly, while finding the promoter, we noticed there was another homologous gene (CaTin1-2) located right in front of CaTin1 in a head-to-head fashion (Fig. 1B). The CaTin1-2 (AF480414) had 158 amino acids and its amino acid sequence showed 58.0% identity and 80.4% similarity to the CaTin1 (AF242731) gene (Fig. 1A) and its nucleotide sequence showed 79.0% identity to the CaTin1 gene. The characteristic feature in CaTin1 and CaTin1-2 found in other proteins is the PLAT domain. Although it is known that some genes containing the PLAT domain code for lipoxygenase, triacylglycerol lipase and lipoprotein lipase in mammalian cells, the function of the PLAT domain has not been studied very well (van Tibeurgh et al. 1996).

To further characterize the genomic organization of these two genes, hot pepper (C. annuum L.) cultivar Bugang genomic DNA was isolated and digested with restriction enzymes, EcoRI, HindIII, XbaI, NcoI, and SalI, and probed with CaTin1 (a), promoter region (b) or CaTin1-2 (c) specific sequences (Fig. 1B). For preparing specific probes of these two genes, we used 5’ region of CaTin1 and 3’ region of CaTin1-2, respectively, to avoid using the highly homologous region, PLAT domain. The promoter region has two XbaI and a SalI restriction sites. Under the high stringency hybridization condi-

---

1 Current address: Donald Danforth Plant Science Center, 975 N Watson Road, St. Louis, MO 63132, U.S.A.
2 Corresponding author: E-mail, khpaek95@korea.ac.kr; Fax, +82-2-928-1274.
CaTin1 and CaTin1-2 share a bidirectional promoter (65°C), EcoRI, HindIII and EcoL restriction enzymes produced one band at the same location and XbaI or SalI restriction enzyme produced bands with different sizes which hybridized with each specific probe (Fig. 1C). These results indicated that each CaTin1 or CaTin1-2 gene is present as a single copy in the pepper genome and no other homologous gene exists in pepper genome.

To examine steady-state transcript levels of CaTin1-2 compared to CaTin1 in various organs of the pepper plant, RNA blot analysis was carried out (Fig. 1D). The CaTin1-2 transcripts accumulated abundantly only in root like CaTin1. Next, the expression pattern of CaTin1-2 gene was monitored in Bugang plants after various treatments (Fig. 2). The plant cultivation, pathogen inoculation and chemical treatment experiments were carried out as described by Park et al. (2002).

The expression pattern of CaTin1-2 gene was monitored in hot pepper cv. Bugang plants after TMV-P0 (avrulent strain) inoculation. A mock inoculation treatment was carried out as a control to cancel out any CaTin1-2 gene expression that might arise from wounding due to rubbing with carborundum. As shown in Fig. 2A, there was almost no detectable accumulation of CaTin1-2 transcripts in mock-inoculated leaves. CaTin1-2 transcripts started to accumulate 48 h after inoculation with TMV-P0 and then was maintained until 72 h post inoculation (h.p.i.). However, CaTin1 transcript was detected from 24 h.p.i. and remained until 72 h.p.i. from the reprobing.
experiment. As a positive control for TMV inoculation, the expression pattern of the CaPR1 gene was also monitored. The induction of the CaPR1 gene was observed beginning from 48 h.p.i., indicating that the expression of CaTin1 gene was induced earlier than that of CaPR1 gene while the CaTin1-2 expression pattern was similar to the CaPR1 expression pattern (Fig. 2A). We also examined whether the CaTin1-2 was turned on during systemic acquired resistance (SAR), and the transcripts corresponding to CaTin1-2 gene started to accumulate in distant uninoculated leaves at 3 d after TMV inoculation (Fig. 2B). The time course of systemic induction of the CaTin1-2 gene was earlier than that of the CaPR1 gene belonging to pathogenesis-related (PR) genes that are known to be induced systemically in tobacco by many pathogens (Sticher et al. 1997) but was very similar to CaTin1.

To investigate whether the TMV-P0-inducible CaTin1-2 gene in plants is also induced during incompatible interaction with other pathogens, pepper leaves were challenged with Xanthomonas campestris pv. vesicatoria (Xcv). RNA blot analysis carried out with the CaTin1-2 specific probe revealed that the transcripts of CaTin1-2 gene accumulated only in ECW-20R carrying the Bs2 resistance gene, suggesting hypersensitive response (HR) specific induction of CaTin1-2 both by viral and bacterial challenges (Fig. 2C).

In pepper leaves sprayed with salicylic acid (SA), expression of CaTin1-2 gene was not induced which is different from CaPR1 transcripts induction, and also CaTin1-2 gene was not induced by methyl jasmonate (MeJA) treatment (Fig. 2D). However, CaTin1-2 gene expression started to increase 2 h after ethylene treatment, and was maintained until 8 h and diminished thereafter. CaTin1-2 gene was also induced slightly by NaCl and significantly by methyl viologen (MV) treatment (Fig. 2E). MV is an inducer of H2O2 (Dodge 1994), which may be the important inducer for the expression of CaTin1-2 gene. Several studies confirmed the importance of SA for the establishment of disease resistance (Dempsey et al. 1999). MeJA and ethylene are plant hormones implicated in stress-related signal pathways (Penninckx et al. 1998). CaTin1-2 was not induced by SA and MeJA but induced only by ethylene. In a previous report, SAR induction was shown to require the signal molecule SA (Gaffney et al. 1993), however, a recent report has shown convincingly that reactive oxygen intermediates (ROI) generated during HR may serve as signals mediating the

![Fig. 2](https://academic.oup.com/pcp/article-abstract/44/5/549/1834755/1834755)
CaTin1 and CaTin1-2 share a bidirectional promoter

Fig. 3 Transcription initiation site determination of the CaTin1-2 by primer extension analysis. DNA sequencing of the corresponding region in CaTin1-2 was performed in parallel using the same oligonucleotide used for primer extension. The arrow designates the transcription start site of CaTin1-2.

SAR (Alvarez et al. 1998). The systemic expression of CaTin1-2 may be related to ROI because CaTin1-2 transcripts could be induced by MV which generates ROI. The expression pattern of CaTin1-2 was very similar to CaTin1 in all the cases except slower expression upon TMV challenge. This almost identical expression pattern may also explain the shared promoter of these two genes.

Analysis of PR gene promoters has led to the identification of the 11-bp ethylene-responsive element TAAGA-GCCGCC, which has been referred as the GCC-box (Ohme-Takagi and Shinshi 1995). This element was shown to be necessary for ethylene response regulation of PR genes in plants. However, the GCC-box has not been found in the promoters of ethylene-regulated genes involved in other ethylene responses such as fruit ripening (Cordes et al. 1989). The elicitor responsive (T)TGAC(C) element, W-box, was first identified within the parsley PRI-1 and PRI-2 promoters and one WRKY transcription factor was known to bind to W-boxes (Rushton et al. 1996). Another WRKY transcription factor, TDBA12, also recognizes a W1-box and is supposed to serve as a component in pathogen-induced signal transduction pathways in plant host cells and regulates expression of certain plant defense genes (Yang et al. 1999). In this report, the CaTin1-2 gene is expressed only in roots under normal conditions and the promoter seems to be responsible for root-specific expression under normal conditions. To date, relatively little data have been published on the regulation of gene expression in roots. cis-acting sequences regulating root-specific expression have not been defined and only a few root-specific promoters were described, and whose activity was rather restricted to some parts of the root (Nitz et al. 2001). The only known cis-acting sequences involved predominantly in root-specific expression are the ocs-elements (Ellis et al. 1987), as-1-elements (Lam et al. 1989) and AT-rich region (Reisdorf-Cren et al. 2002), and CaTin1-2 promoter has two AT-rich regions.

To determine the precise transcription initiation site of the CaTin1-2 gene, a 5'-GTTTTCCGATCATTTCCCAACCA-3', which is complementary to the sense strand sequence of the CaTin1-2 cDNA was radiolabeled at their 5' terminus with T4 polynucleotide kinase and [γ-32P]ATP. Approximately 100 fmol of the labeled primers were hybridized with 10 μg of total RNA which were isolated from leaves tissues 4 h after ethylene treatment. After reverse transcription, the reaction products were electrophoresed through a 6% denaturing polyacrylamide gel and visualized by autoradiography. Through the primer extension experiments, the transcription start site of CaTin1-2 was verified. The location of CaTin1-2 transcription start site was 955 bp from the transcription start site of CaTin1-2 homologue, CaTin1 (Fig. 3). The promoter of CaTin1-2 has four ethylene responsive GCC-boxes, two root-specific elements (AT-rich regions) and an elicitor-induced WRKY transcription factor binding W1-box (Fig. 4A). Among four GCC-boxes, only one box is in the antisense direction and the others are in the sense direction. It also has TATA-box (data not shown). In order to characterize further the sequences involved in CaTin1-2 transcriptional regulation, we generated sets of deletions in full-length promoter region of CaTin1-2 and connected those with GUS coding gene. The p+a4Tin1 and p+a95Tin1 constructs contain the full-length promoter of CaTin1-2 and p-981Tin1 construct contains CaTin1 full-length promoter (Fig. 4A). Because CaTin1-2 gene was induced by TMV inoculation and ethylene, the tobacco transgenic plants with deletion constructs of CaTin1-2 promoter were tested for GUS activities 48 h after TMV, 8 h after ethylene treatment and 24 h after MV treatment. For each assay, 112.5 μl of 4-methylumbelliferyl β-D-glucuronide solution was pre-incubated in a microtube in a water bath for 5 min at 37°C. Then, 75 μl of cell extract in GUS/LUC buffer (0.1 M KPO4, pH 7.8, 2 mM Na2EDTA, 2 mM DTT and 5% glycerol) was added and incubated at 37°C. After 30 min or 60 min, 50 μl aliquot was removed from each assay tube and transferred to the test tube containing 1 ml stop solution (0.2 M Na2CO3). The progress of the reactions was quantitatively assessed by measuring the stopped reactions under emission wavelength of 455 nm (Maliga et al. 1995). When the transgenic tobacco plants of deletion constructs of CaTin1-2 promoter were inoculated with TMV, they had high GUS activity compared to the promoterless GUS construct (p-GUS) as long as they had a W1-box and they had similar activity to opposite directional promoter, p-981Tin1 construct (Fig. 4B). Even though it seems to need participation of other cis-element(s) for the full activation of CaTin1-2 promoter, W1-box is certainly giving an effect for the TMV response of CaTin1-2. Although CaTin1 and CaTin1-2 expression patterns and time were very similar, the expression of CaTin1 was earlier than that of CaTin1-2 upon TMV infection (Fig. 2A) and that may be simply caused by the degree of...
CaTin1 and CaTin1-2 share a bidirectional promoter 553

accessibility to the W1-box. When they did not have any GCC-box, they hardly showed the GUS activity and if they had one GCC-box, they had lower GUS activity upon ethylene treatment. However, in case of over three GCC-boxes they had higher GUS activity. Thus, the GUS activities of deletion promoters upon ethylene response were dependent on the GCC-boxes.
box (Fig. 4C). With respect to MV response, even though there is no known MV-responsive element so far, the full size promoter of CaTin1-2 showed highest GUS activity compared to the other deletion constructs (Fig. 4D). From the histochemical staining experiments of GUS activity, the GUS activity of CaTin1-2 promoter was not distributed in all the root parts but was mainly in the apical meristem of root like CaTin1 activity distribution (data not shown).

The CaTin1-2 promoter was bidirectional; however, with the exception of the promoter of oleosin and methionine sulfoxide reductase genes in Brassica, bidirectional type promoter has been hardly reported in plants (Keddie et al. 1994). In human and animal systems, genes encoding for DNA-damage related enzyme (Ame et al. 2001), protein related to energy metabolism (Chinenov et al. 2000) and histone (Huh et al. 1991), which are some important and/or urgency-related proteins during the life time, have bidirectional promoters. Thus CaTin1-2 and CaTin1 sharing the promoter sequence in a bidirectional fashion may be regulated at the same time in urgent situations such as pathogen infection in hot pepper plants.

Acknowledgments

This work was funded by a Crop Functional Genomics Center of the 21st Century Frontier Research Program grant (CGI2122) and a G7 grant from the Korea Ministry of Science and Technology and a grant from the Plant Signaling Network Research Center, Korea Science and Engineering Foundation.

References


