Characteristics of the promoters derived from the single-stranded DNA components of *Milk vetch dwarf virus* in transgenic tobacco

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Predicted promoter regions of *Milk vetch dwarf virus* (MDV) components (C1–C11) were isolated and fused with a β-glucuronidase (GUS) reporter gene and the characteristics of the promoters were examined. In transgenic tobacco calli, promoters of MDV C4 (encoding a cell-cycle link protein), C5 and C7 (both encoding unknown proteins), C6 (encoding a nuclear-shuttle protein) and C8 (encoding a movement protein) generated a stronger level of GUS expression than the *Cauliflower mosaic virus* 35S RNA promoter (P35S). In leaves of transgenic tobacco plants, the promoters of C5 and C8 conferred a level of GUS activity comparable to that of P35S. Histochemical GUS analysis showed that the promoters of C4–C9, the latter encoding a capsid protein, were active in phloem and meristematic tissue. The promoter of C8 was also active in mesophyll and cortex cell types. A low level of activity was found for the promoters of C11, which encodes a master replication-initiator protein (Rep), and C1, C2, C3 and C10, which encode additional Reps, in both transgenic tobacco calli and plants.

**INTRODUCTION**

*Milk vetch dwarf virus* (MDV) is a member of the family *Nanoviridae* (Gronenborn, 2004; Vetten et al., 2004). The family *Nanoviridae* consists of two genera (Vetten et al., 2004). Whilst *Banana bunchy top virus* (BBTV; Harding et al., 1991) is currently the only member of the genus *Babuvirus*, the genus *Nanovirus* includes *Faba bean necrotic yellows virus* (Katul et al., 1993), *Subterranean clover stunt virus* (SCSV; Chu & Helms, 1988) and MDV. *Coconut foliar decay virus* (CFDV; Randles & Hanold, 1989) is an unassigned species in the family. MDV is transmitted by *Aphis craccivora* in a persistent manner and causes yellowing and dwarfing of several leguminous crops, including Chinese milk vetch (*Astragalus sinicus* L.), broad bean (*Vicia faba* L.), pea (*Pisum sativum* L.) and soybean (*Glycine max* (L.) Merr.). Some solanaceous plants have also been reported to be susceptible to MDV (Inoue et al., 1968). Particles of MDV were observed in the phloem cells of MDV-infected broad bean leaves (Okita et al., 1975). Eleven circular single-stranded DNA (ssDNA) components have been isolated from the N isolate of MDV (Sano et al., 1998; Timchenko et al., 2000). Each component is approximately 1 kb in size, potentially encodes one large gene in the virion sense and contains a potential stem–loop (SL) structure in the non-coding region. C11 (DNA-R) encodes the master Rep (M-Rep), which is the replication-initiator protein (Rep) for all other genomic DNAs (Timchenko et al., 2000). Based on the sequence similarity between proteins of different nanoviruses, it is supposed that C4 (DNA-C), C6 (DNA-N), C8 (DNA-M) and C9 (DNA-S) encode the cell-cycle link protein (Clink), the nuclear-shuttle protein (NSP), the movement protein (MP) and the capsid protein (CP), respectively (Vetten et al., 2004). The functions of the proteins encoded by C5 (DNA-U1) and C7 (DNA-U2) are unknown. C1, C2, C3 and C10 encode Reps, but appear to be satellite-like DNAs.
The promoter activity associated with some members of the family Nanoviridae, BBTV, SCSV and CFDV, has been characterized. In transgenic tobacco and banana plants, the promoters derived from the BBTV C1–C6 intergenic regions generally gave weak, tissue-specific expression patterns restricted to phloem-associated cells and at least one of them was highly expressed in actively dividing, undifferentiated cell types (Dugdale et al., 1998, 2000). In SCSV, the promoter activity varies between the seven components and appears to be primarily vascular-associated (Surin et al., 1998). SCSV C4 (DNA-N) and C7 (DNA-U1) showed substantial activity in transgenic tobacco plants. SCSV C2 and C6, which encode satellite Reps, had almost undetectable activity. SCSV C1 (DNA-M) conferred weak vascular expression, but its activity increased in callus tissue. The promoter associated with the intergenic region of CFDV had weak phloem-specific activity in transgenic tobacco and substantial activity in Escherichia coli (Rohde et al., 1995; Hehn & Rohde, 1998).

In this study, the activities of predicted promoter sequences derived from MDV C1–C11 have been assessed to understand the control of gene expression in MDV. Bacterial cells and tobacco plants were transformed with chimeric genes consisting of MDV-derived promoters fused to a β-glucuronidase (GUS) reporter gene to examine their expression profiles. The results indicate that expression of the individual components is differentially regulated.

**METHODS**

**Isolation of MDV-derived promoter sequences.** MDV genomic nucleic acid was purified from MDV-infected pea leaves essentially as described previously (Sano et al., 1998). The genomic organization of MDV C1–C11 and the predicted promoter regions are illustrated in Fig. 1(a). cis-acting elements of the viral promoter sequences were analysed by searching the database PLACE (Higo et al., 1999) for a plant cis-element motif. The predicted promoter fragments were isolated by PCR using the primers listed in Table 1. Each primer contains a restriction site in the 5’ flanking region. The sites are shown with the names of primers in Table 1. The products amplified from MDV C1, C2, C3, C8 and C10 were cloned in pBluescriptII SK (Stratagene) following digestion with HindIII and BamHI. Those of MDV C4, C5, C6, C7, C9 and C11 were introduced by digestion with PstI and BamHI. Nucleotide sequences of these fragments were confirmed by using an Applied Biosystems 373A DNA sequencer.

Deletions of the MDV C5 and C8 promoter fragments were generated by PCR. C5d1, d2, d3 and d4 were amplified by using the sense primers PT5-D1 (5’-AAGTCGATATTTATTATTATTATTATTATCTC-TAGG-3’), PT5-D2 (5’-AATGCCGATTGCGTGCTGATGCC-3’), PT5-D3 (5’-AATGGCGATGGGCGGCCGTTGATTTTAC-3’), PT5-D4 (5’-AATGGCGATGGGCGGCCGTTGATTTTAC-3’), and PT5-D5 (5’-AATGGCGATGGGCGGCCGTTGATTTTAC-3’), respectively, which all contain a HindIII site in the 5’ flanking region, and the antisense primer C8-PT(−)BamHI (Table 1). The amplified products were cloned in plasmid pGEM-T (Promega).

**GUS reporter-gene construction.** The promoter fragments of C1, C2, C3, C8 and C10 were each excised from pBluescriptII SK by digestion with HindIII and BamHI, whereas those of C4, C5, C6, C7 and C11 were excised by digestion with SalI and BamHI. These promoter fragments were cloned in the binary vector pBI101.3 (Clontech) as transcriptional GUS gene fusions. The resulting chimeric plasmids were named PMC1–PMC11::GUS, respectively. The plasmid pBI121 (Clontech), referred to as P35S::GUS in this report and containing the Cauliflower mosaic virus 35S RNA promoter, was included as a positive control. Deletions of the MDV C5 and C8 promoter fragments were excised from the vector pGEM-T by digestion with HindIII and BamHI and cloned into pBI101.3.

Plasmid DNA was extracted from overnight cultures of E. coli JM109 (TOYOBO) by using a Quantum Prep Kit (Bio-Rad) according to the supplier’s recommendations. All promoter fusions and the control were introduced into Agrobacterium tumefaciens strain LBA4404 (Ooms et al., 1982; Hoekema et al., 1983) by direct transformation.

**Transformation of tobacco plants.** Tobacco (Nicotiana tabacum cv. Samsun NN) plants were co-cultivated with Agrobacterium by the leaf disc-infection method (Horsch et al., 1985) and transformants were selected on MS medium (Murashige & Skoog, 1962) supplemented with 100 mg kanamycin l−1 and 500 mg carbenicillin l−1. Regenerated plants were analysed for integration of the promoter::GUS fusion genes into the plant genome by PCR. For detection of the GUS region, a sense primer sequence from the GUS coding region, 5’-GCAACGTCTGTTATCACCC-3’ (corresponding to nt 194–211), and an antisense primer sequence from the nopaline synthase gene terminator region, 5’-TCTAGTAACATAGTGA-TGACA-3’ (corresponding to nt 2097–2079), were used. For the promoter region, each of the primer sets in Table 1 was used. The reactions were run with an initial denaturation at 94 °C for 1 min, then 94 °C for 30 s, 55 °C for 30 s and 72 °C for 1 min for 30 cycles, and a final extension at 72 °C for 8 min.

**Measurement of GUS activity.** GUS activity was assayed by the method of Kosugi et al. (1990). Calli or leaf discs from transgenic tobacco plants were homogenized in lysis buffer [50 mM sodium phosphate (pH 6–8), 10 mM EDTA, 10 mM 2-mercaptoethanol, 0·1% Triton X-100 and 0·1% sarcosyl] in an Eppendorf tube with a glass rod and carborundum (600 mesh; Nakalai Tesque). The homogenate was centrifuged at 10 000 g for 15 min and the supernatant was assayed for GUS activity in the presence of 20% methanol. Fluorescence levels were determined by using a Hitachi F-2500 spectrometer.

**Histochemical analysis.** Leaf discs, longitudinal half-cut stems, including side buds, and intact roots from the transgenic tobacco plants containing each promoter::GUS construct were subjected to GUS staining. Tissue sections from transgenic tobacco plants were cut at 80–100 μm with a microslicer (model DTK–1000; Dosaka). Histochemical staining for GUS activity was performed at 37 °C as described previously (Ohshima et al., 1990) with a modified reaction mixture; 50 mM phosphate buffer (pH 7·0) containing 1 mM 5-bromo-4-chloro-3-indolyl glucuronicide (X-Gluc), 5% methanol, 10 μg cycloheximide ml−1 and 1 mM dithiothreitol. The reaction was stopped by the addition of ethanol.
RESULTS

Activity of MDV-derived promoters in tobacco calli and plants

Activities of MDV-derived promoters were assessed in dividing cells. The GUS activity in eight individual, regenerated, kanamycin-resistant calli at 20 days after selection was assayed by a fluorometric quantification method. Fig. 2 shows levels of GUS activity conferred by each MDV-derived promoter in kanamycin-resistant calli. The levels of GUS activity in calli containing PMC4::GUS, PMC5::GUS, PMC6::GUS, PMC7::GUS, PMC8::GUS, PMC9::GUS and P35S::GUS were 7.08–26.30, 19.20–52.50, 4.58–25.40, 13.30–39.20, 10.00–37.90, 2.50–12.50 and 2.13–7.63 nmol 4-methyl umbelliferone (4-MU) (mg fresh weight)\(^{-1}\) h\(^{-1}\), respectively. In contrast, low activity was generated by the promoters of DNA-R and additional Rep-encoding DNAs. The range of GUS activities conferred by PMC1::GUS, PMC2::GUS, PMC3::GUS, PMC10::GUS and PMC11::GUS were 0.083–0.29, 0.21–0.46, 0.17–0.38, 0.33–1.00 and 0.29–0.88 nmol 4-MU (mg fresh weight)\(^{-1}\) h\(^{-1}\), respectively. When the same constructs were delivered to the pea and tobacco leaves according to the protocol for the Biolistic Particle Delivery System (Bio-Rad) and GUS activity was assessed histochemically, transient GUS expression of the promoters of DNA-R and additional Rep-encoding DNAs was found (see Supplementary Fig. S1, available in JGV Online).

Fig. 1. Schematic representations of the proposed organization of (a) the circular ssDNA genome of MDV and predicted promoter region and (b) MDV promoter fragments analysed. See text for explanation of cis elements.
Transgenic tobacco plants containing promoter::GUS constructs were regenerated from kanamycin-resistant calli. Between 20 and 27 independent lines of transgenic tobacco plants were established for each construct and PCR was used to confirm that each plant contained the GUS gene. The level of GUS activity in mature leaves of 1-month-old transgenic lines with PMC4::GUS, PMC5::GUS and PMC8::GUS was comparable to that of P35S::GUS plants. PMC6::GUS and PMC7::GUS conferred almost half the level of activity generated by P35S::GUS. The

<table>
<thead>
<tr>
<th>Component (GenBank accession no.)</th>
<th>Primer name</th>
<th>Sequence</th>
</tr>
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<tbody>
<tr>
<td>MDV C1 (AB000920)</td>
<td>C1PT(+)HindIII</td>
<td>5'-GGGAAGCTTATATGTTATGTGGAGG-3'</td>
</tr>
<tr>
<td>MDV C2 (AB000921)</td>
<td>C2PT(+)HindIII</td>
<td>5'-GGGAAGCTTGGCACCAGCATCTGAGGAGA-3'</td>
</tr>
<tr>
<td>MDV C3 (AB000922)</td>
<td>C3PT(+)HindIII</td>
<td>5'-GGGAAGCTTGACCCAGGACATACTG-3'</td>
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<tr>
<td>MDV C4 (AB000923)</td>
<td>C4PT(+)PstI</td>
<td>5'-AACCTGCAGAATTAAATACCTTTACAT-3'</td>
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<tr>
<td>MDV C5 (AB000924)</td>
<td>C5PT(+)PstI</td>
<td>5'-AACCTGCAGAATTAAATACCTTTACAT-3'</td>
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<tr>
<td>MDV C6 (AB000925)</td>
<td>C6PT(+)PstI</td>
<td>5'-AACCTGCAGAATTAAATACCTTTACAT-3'</td>
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<tr>
<td>MDV C7 (AB000926)</td>
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<tr>
<td>MDV C8 (AB000927)</td>
<td>C8PT(+)PstI</td>
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<tr>
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<tr>
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<td>5'-GGGAAGCTTGAGATGATTATAGTTTG-3'</td>
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<tr>
<td>MDV C11 (AB027511)</td>
<td>C11PT(+)PstI</td>
<td>5'-AACCTGCAGAATTAAATACCTTTACAT-3'</td>
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Table 1. Sequences of the primers used in this study

Fig. 2. Levels of GUS activity conferred by MDV-derived promoters in kanamycin-resistant (KmR) calli. The level of GUS activity in KmR calli containing each promoter::GUS fusion construct was measured as described in Methods. Means±SD from eight independent experiments are shown. The ratio of the mean value conferred by each promoter to that by P35S is shown at the bottom of the figure. Data were subjected to Tukey’s multiple-comparisons procedure. There is a significant difference between values marked c and d at P<0.05 and between values marked with other letters at P<0.01. FW, Fresh weight.
level of GUS expression provided by PMC9 was low. PMC1::GUS, PMC2::GUS, PMC3::GUS, PMC10::GUS and PMC11::GUS plants showed little or no expression of GUS (Fig. 3).

Tissue-specific expression of MDV-derived promoters in tobacco plants

The expression profiles of MDV-derived promoters in tobacco plants were studied further by using histochemical GUS assays. Tissue-specific GUS expression patterns in leaf discs, longitudinally cut stems, including side buds, and intact roots from 20 independent transgenic tobacco lines for each promoter::GUS construct were examined after overnight GUS staining and are summarized in Table 2. PMC1, PMC2, PMC3, PMC10 and PMC11, which are the promoters for the four additional Rep-encoding DNAs and DNA-R, respectively, conferred variable GUS expression in the meristem and the vascular bundle of 1-month-old transgenic tobacco plants. In contrast, PMC4, PMC5,

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Table 2. Expression patterns in transgenic tobacco tissues

Summary of the GUS expression patterns observed in 20 independent transgenic lines obtained with each MDV-derived promoter::GUS fusion construct after overnight staining at 37°C. ±, Weak or variable expression in this tissue across the 20 transgenic lines examined.

<table>
<thead>
<tr>
<th>Component</th>
<th>Shoot apex</th>
<th>Leaf</th>
<th>Stem</th>
<th>Root</th>
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<tr>
<td></td>
<td></td>
<td>Vascular bundle</td>
<td>Mesophyll</td>
<td>Pith</td>
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<td>MC1</td>
<td>±</td>
<td>–</td>
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<td>MC2</td>
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<td>MC6</td>
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<td>MC7</td>
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<td>MC8</td>
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<td>MC9</td>
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<td>MC10</td>
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<td>MC11</td>
<td>±</td>
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PMC6, PMC7, PMC8 and PMC9 generated intense GUS staining in the shoot apex, the root tip and the vascular bundle of leaves. Intensity of staining in the leaf discs of each promoter::GUS plant seemed to correlate to the level of quantitative GUS activity (Fig. 3). PMC8::GUS conferred weaker GUS expression in vascular bundles than PMC5::GUS plants, but was expressed universally in most cell types. To study the expression of GUS more precisely, sections of shoot apex and fully expanded leaves containing midribs made from three lines each of 2-month-old PMC1–PMC11::GUS plants were examined. After staining for 2–8 h, strong GUS activity was observed in the meristem.
and vascular bundle in the longitudinal section of the shoot apex from PMC4, PMC5, PMC6, PMC8 and PMC9::GUS plants. A typical expression pattern is shown in Fig. 4[a (i)]. The section of shoot apex from PMC1, PMC2, PMC3, PMC10 and PMC11::GUS plants showed no or weak GUS expression in the meristem and vascular bundle of the top leaf after staining for 18–24 h. Fig. 4[a(ii)] shows one of the sections from PMC10::GUS plants. GUS expression in the cross-sections of midrib from fully expanded leaves from PMC4, PMC5, PMC6, PMC8 and PMC9::GUS plants was observed specifically in the phloem. A typical specimen is shown in Fig. 4[b(i)]. GUS staining was not detected in the sections of mature leaves from PMC1, PMC2, PMC3, PMC10 or PMC11::GUS plants after staining for 24 h in this experiment (data not shown). In PMC4, PMC5, PMC6 and PMC9::GUS plants, GUS expression was limited to the vascular system in the leaves and roots [representative examples are shown in Fig. 4b(ii, iii)]. In PMC8::GUS plants, GUS staining also occurred in the mesophyll of the leaves and cortex of stems and roots [Fig. 4b(iv, v)].

**Effects of the deletions of PMC5 and PMC8 in tobacco plants**

To identify regions that determine the character of the promoters, deletions of PMC5 and PMC8 were made (Fig. 5a) and promoter activities were assessed in transgenic tobacco leaves. However, when leaf discs from all transgenic tobacco lines for each deletion promoter::GUS construct were examined after overnight GUS staining, they exhibited tissue-specific expression similar to their original promoter (data not shown). Results of quantitative GUS analysis are shown in Fig. 5(b). There is a statistically significant difference in strength between the C5d1 promoter and PMC5 at \( P < 0.01 \). GUS activity associated

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![Diagram](http://vir.sgmjournals.org)

**Fig. 5.** PMC5 and PMC8 intergenic deletions and their activity in transgenic tobacco leaves. (a) Deletions of PMC5 and PMC8. (b) Levels of relative GUS activity conferred by MDV-derived promoters in transgenic tobacco leaves. Leaf discs were cut from the fully expanded upper leaves of 1-month-old regenerated transformants. Mean GUS activity, ratio of mean value conferred by each promoter to that by PMC5 or PMC8 and numbers of plants used are shown at the bottom of the figure. Each dot shows the GUS value of an individual transgenic plant. Bar, mean value. Data were subjected to Tukey’s multiple-comparisons procedure. There is a significant difference between values marked a and b at \( P < 0.05 \) and between values marked a’ and b at \( P < 0.01 \).
with C5d2, C5d3 and C5d4 was comparable to that of PMC5. No significant differences in activity were found between the promoters of C8d1, C8d4, C8d5 and PMC8.

**DISCUSSION**

This study presents a characterization of promoter activity associated with the intergenic regions of the 11 components of MDV. It has been shown that some predicted promoter fragments of non-Rep-encoding DNAs conferred activity comparable to that of P35S in transgenic tobacco plants and activity stronger than that of P35S in transgenic calli. In contrast, promoters of the Rep-encoding DNAs generated low levels of activity in transgenic tobacco plants.

The low level of promoter activity by Rep-encoding DNAs in transgenic tobacco plants and calli might have been expected, given the previous results for other members of the family *Nanoviridae*. BBTV C1 (DNA-R) and SCSV C2 and C6, which encode satellite Reps, have been reported to confer negligible activity in assessments of transient and stable expression using tobacco cells and plants (Dugdale et al., 1998; Surin et al., 1998), whereas BBTV C1 and SCSV C2 have been shown to be able to replicate autonomously (Chu et al., 1995; Horser et al., 2001). This indicates that the basal activity of Rep promoters is extremely low, but sufficient to express Rep and initiate DNA replication.

The MDV-derived, non-Rep promoters conferred a higher level of GUS expression in calli than in plants, whereas P35S conferred the same level. This is consistent with the reported activities of non-Rep promoters of BBTV and SCSV. Dugdale et al. (1998) pointed out the possibility that the CATGACGTCA sequence in BBTV, which contains the ASF1 motif (TGACG; Lam et al., 1989; Benfey & Chu, 1990) and a hexamer motif characteristic of plant histone promoters (ACGTCA; Mikami et al., 1987; Nakayama et al., 1992; Morozov et al., 1994), contributed to the strong promoter activity in undifferentiated, actively dividing cell types. The TGACGTCA sequence has been reported as a palindromic C box that is recognized by two bZIP proteins with a zinc-finger motif, STF1 and STF2, from soybean apical hypocotyl (Cheong et al., 1998). This sequence is found in all MDV-derived non-Rep promoters and MDV-C11 (Fig. 1b). However, PMC11 conferred weak promoter activity in transgenic tobacco plants, as well as other additional Reps (Figs 2 and 3). Furthermore, the deletion of the palindromic C element, as well as the SL domain, in the deletions of PMC5 and PMC8 had no significant effect on promoter activity in transgenic tobacco plants (Fig. 5).

Dof proteins are members of a major family of transcription factors unique to plants and have a similar DNA-binding specificity for the sequence AAAG or CTTT (Yanagisawa, 2002). Dof proteins have diverse roles in gene expression associated with plant-specific phenomena. A tobacco Dof protein, NTBBF1, is involved in auxin-inducible expression of a plant oncogene, rolB, in apical meristem and vascular tissues (Baumann et al., 1999). An investigation of cis elements in the predicted promoter regions of MDV by PLACE showed four to eight Dof domains and zero to four NTBBF1 domains 200 bp upstream of each TATA box in the non-Rep promoters, and two to four Dom domains scattered in the Rep promoters (Fig. 1b). There is a possibility that these Dof domains determine the difference in expression profiles between non-Rep and Rep promoters.

Promoters derived from non-Rep-encoding MDV DNAs were active in phloem and meristematic tissue. Expression of PMC8 was not limited to the phloem and meristem, but was also observed in mesophyll and cortex cell types. Of other nanovirus promoters derived from DNA-M, BBTV C4 did not appear to be confined to the leaf vascular tissues, with visible green fluorescent protein expression in stomata and mesophyll cells (Dugdale et al., 2000), and SCSV C1 conferred a weak vascular expression (Surin et al., 1998). The factor responsible for the tissue-specific expression conferred by MDV promoters could not be determined in this study.

PMC5 differed in terms of the strength of the activity it conferred in transgenic tobacco plants and calli, despite its similarity to PMC4 and PMC7. PMC5 has a unique 90 bp region downstream of the TATA box. This region contains the CT-rich sequence, TCTTCTCTTTTCACAAACAAC, near the presumed transcription-initiation site. This sequence resembles the CT-rich sequences in 5’-untranslated leaders of genes for thylakoid proteins, which are essential for transcription (Bolle et al., 1994) and may function as a plant-specific regulatory DNA sequence. The result that PMC5 conferred almost no activity in *A. tumefaciens* (see Supplementary Fig. S2, available in JGV Online) also suggests that these CT-rich sequences function as a plant-specific initiation region.

In the experiment with the deletion of PMC5, C5d1 (495 bp) showed a high level of promoter activity compared to PMC5 (532 bp) (Fig. 5b). From this result, a 37 bp region from the 5’ end of PMC5 may contain a downregulatory element. Dugdale et al. (1998) examined the transient activity in tobacco cells with deletions of BBTV C6 and obtained similar results. However, common sequences were not found in these regions between BBTV and MDV.

PMC9 conferred a relatively low level of activity in transgenic plants, compared with other promoters derived from non-Rep-encoding MDV DNAs. CP will be required in large amounts later in the infection cycle. There is a possibility that other viral proteins, which are generated earlier in the replication of MDV, activate expression of the CP promoter. Studies with the family *Geminiviridae*, a related ssDNA virus group, have revealed that promoter activity is influenced by the interaction of virus-encoded gene products (Hanley-Bowdoin et al., 2000). In *Tomato golden mosaic virus*, which is a member of the genus *Begomovirus*, the product of the AL2 gene is necessary for efficient...
transcription from the CP promoter (Sunter & Bisaro, 1991). No information is available concerning which elements are involved in MDV CP expression, yet it is assumed that some regulatory elements may control a transcriptional cascade to warrant an efficiently timed gene expression in the family Nanoviridae.

As the activity levels of MDV non-Rep promoters were higher than that of P35S in transgenic tobacco calli, these promoters would be useful for foreign gene expression in proliferating tissues. The GUS expression levels in individual transgenic plant lines with MDV-derived promoter(s): GUS varied greatly (Fig. 3). The observed variation in expression is probably due to a positional effect and a different number of integrated copies of the transgenes in transgenic plants (Hobbs et al., 1990, 1993; Peach & Velten, 1991). Nevertheless, PMC8 confers efficient gene expression and may serve as an alternative to P35S, which has been used widely for genetic manipulation (Shirasawa-Seo et al., 2005).

As each MDV-derived promoter has unique expression characteristics, further study of MDV-derived promoters will provide valuable understanding of not only the regulatory elements that determine specificity, but also the mechanisms that regulate nanovirus gene expression in host plants.

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