Evidence for interaction between the 2a polymerase protein and the 3a movement protein of *Cucumber mosaic virus*

Min Sook Hwang, Sang Hyon Kim, Jeong Hyun Lee, Jung Myung Bae, Kyung Hee Paek and Young In Park

**Correspondence**
Young In Park
yipark@korea.ac.kr

School of Life Sciences and Biotechnology, Korea University, Anam-dong 5-ga, Seongbuk-gu, Seoul 136-701, Korea

The genome of *Cucumber mosaic virus* consists of three single-stranded RNA molecules, RNAs 1, 2 and 3. RNAs 1 and 2 encode the 1a and 2a proteins, respectively, which are necessary for replication of the viral genome and have been implicated in movement of the viral RNAs in plants. The 3a movement protein (MP), encoded by RNA 3, is essential for transferring the RNA genomes from infected cells to adjacent cells across the plasmodesmata. Far-Western analysis demonstrated that bacterially expressed 2a polymerase protein directly interacted with the MP. Interaction was confirmed in a yeast two-hybrid assay, and co-immunoprecipitation analysis showed that the MP interacted only with the 2a polymerase protein. A yeast three-hybrid assay showed that the 1a–2a protein interaction relevant for replicase complex formation was not affected by the MP. Although the MP has no affinity for the 1a protein, it interacted indirectly with the 1a protein via the 2a polymerase protein. These results suggest that the replicase complex may be involved in movement through its interaction with the MP.

**INTRODUCTION**

*Cucumber mosaic virus* (CMV) is the type member of the genus *Cucumovirus* in the family *Bromoviridae*. CMV particles have an icosahedral shape and contain a tripartite, positive-sense RNA genome, designated RNAs 1–3 (Palukaitis *et al*., 1992). CMV RNAs 1 and 2 each encode one protein involved in replication of the viral genome, designated 1a protein and 2a polymerase protein, respectively (Palukaitis *et al*., 1992; Buck, 1996). RNA 1, containing sequence motifs conserved in methyltransferases (Mi *et al*., 1989; Mi & Stollar, 1991; Rozanov *et al*., 1992) and helicases (Hodgman, 1988; Gorbalenya *et al*., 1989; Habili & Symons, 1989), and RNA 2, encoding the polymerase, have been shown to be associated in a membrane-bound RNA-dependent RNA polymerase (RdRp) (Hayes & Buck, 1990) designated the CMV replicase. The active CMV replicase consists of both 1a and 2a polymerase proteins, as well as one or more host factors. This RdRp participates in the synthesis of both double-stranded and single-stranded RNA and has been isolated and purified from infected tobacco tissue (Hayes & Buck, 1990). RNA 2 has an additional open reading frame (ORF) encoding a protein called 2b (Ding *et al*., 1994), which participates in host-specific virus accumulation, suppression of post-transcriptional gene silencing and virulence determination (Lucy *et al*., 2000). RNA 3 encodes the 3a movement protein (MP), which is crucial for movement of viral RNA from cell to cell (Ding *et al*., 1995). The MP also binds to single-stranded nucleic acid *in vitro* (Li & Palukaitis, 1996; Vaquero *et al*., 1997). RNA 4 is subgenomic mRNA derived from the 3′ half of RNA 3 and is involved in the synthesis of viral coat protein (CP) (Schwinghamer & Symons, 1975). It has been reported that RNAs 1, 2 and 3 are essential for the systemic infection of plants (Rao & Francki, 1982; Palukaitis *et al*., 1992). In addition, all five proteins encoded by the three genomic RNAs of CMV affect the movement of CMV (reviewed by Palukaitis & Garcia-Arenal, 2003).

CMV MP localizes to plasmodesmata and to parietal sieve elements in the phloem (Blackman *et al*., 1998). The 2a polymerase protein and the 1a protein predominantly co-localize to vacuolar membranes (the tonoplast), and negative-strand CMV RNAs are also associated with the tonoplast (Cillo *et al*., 2002). Thus, it appears that the 2a polymerase protein and MP have independent roles and a different subcellular distribution. However, CMV 2a polymerase protein and MP both affect the rate of movement of CMV in zucchini squash, although these two proteins function independently with the host to facilitate virus movement (Choi *et al*., 2005).

Studies of other viruses have indicated that the 2a polymerase protein has a similar subcellular distribution to the MP and is involved in movement. In *Brome mosaic virus*...
(BMV), a member of the genus Bromovirus in the family Bromoviridae and taxonomically related to CMV, systemic infection in barley plants was inhibited by deletions in 2a proteins that supported strong RNA replication in protoplasts (Traynor et al., 1991). The 1a and 2a proteins co-localized on plant endoplasmic reticulum, the site of nascent BMV RNA synthesis (Restrepo-Hartwig & Ahlquist, 1996). As in plant systems, the distribution of RNA 3 is associated with the sites of 1a and 2a protein accumulation in yeast cells co-expressing the 1a and 2a proteins and RNA 3 (Restrepo-Hartwig & Ahlquist, 1999). In Tobacco mosaic virus (TMV), the type member of the genus Tobamovirus in the family Tobamoviridae, a considerably different system from CMV or BMV is found, where the helicase domain of the replicate coding region has been shown to be involved in cell-to-cell movement (Hirashima & Watanabe, 2001, 2003).

Since CMV 2a polymerase protein may play roles other than in viral RNA synthesis, we considered that it might regulate other virally encoded proteins, most simply by direct protein–protein interactions. In this study, we assayed viral protein interactions using yeast two-hybrid and far-Western techniques. The results indicated that CMV MP interacts directly with the 2a polymerase protein and indirectly with the 1a protein via the 2a polymerase protein, suggestive of an additional regulatory mechanism that exploits interactions among viral proteins to promote virus propagation.

METHODS

Recombinant proteins. cDNAs specifying ORFs of the As strain of CMV (CMV-As) [Park et al., 1990; GenBank accession nos AF003667 (RNA 2) and AF013291 (RNA 3)] were inserted into pGEX-KG and transformed into the Escherichia coli strain BL21. Glutathione S-transferase (GST) fusion proteins were purified as described previously (Kim et al., 2002).

Far-Western blotting. Labelling of GST-fused 2a polymerase protein purified from E. coli was done according to Kim et al. (2002). GST fusion protein (100 ng) was incubated in kinase buffer (20 mM Tris/HCl, pH 7.5, 100 mM NaCl, 1 mM dithiothreitol, 12.5 mM MgCl2) including 1 μCi [γ-32P]ATP μl−1 for 30 min. Unincorporated, free [γ-32P]ATP was removed by chromatography through a Sephadex G-25 spin column equilibrated in 20 mM Tris/HCl pH 8.0, 1 mM EDTA, 100 mM NaCl, 0.1% NP-40 and 1 mM PMSF. SDS-PAGE was performed as described by Schagger & Jagow (1987). Proteins in the SDS-polyacrylamide gel were transferred to nitrocellulose membrane in buffer containing 25 mM Tris/195 mM glycine at 250 mA for 1.5 h at 4°C and the following steps were performed at this temperature. The membrane was placed in 100 ml binding buffer (20 mM HEPES/KOH, pH 7.7, 75 mM KCl, 0.1 mM EDTA, 2.5 mM MgCl2, 1 mM DTT, 0.05% NP-40) for 30 min, soaked in blocking buffer (5% BSA in binding buffer) for 4 h and hybridized with 32P-labelled (10 5 c.p.m. ml−1) GST-2a fusion protein in interaction buffer (1% BSA in binding buffer) overnight. The membrane was rinsed in interaction buffer for 3 h with three changes of buffer and exposed to X-ray film for 4–12 h.

Yeast plasmids. The CMV 2a polymerase ORF was amplified by PCR using previously described primers (Kim et al., 2002), digested with Smal and PstI and ligated to Smal/PstI-digested pAS2-1, which harbours the GAL4 binding domain (BD) (Clontech). The ORF for CMV 1a protein (Kim et al., 2002), 2b protein, MP and CP were also amplified using specific primers as follows. For the 2b ORF: nt 2414–2421, 5'-GAGACCCGGGGGATATGGAATGGAACAGGG-3' and nt 2723–2746, 5'-GAGAGAGCTCACAAGAACGCTTGACC-3'. For the MP ORF: nt 123–140, 5'-GAGAGGGGGCAT-ATGGCTTTCCAAGTACC-3' and nt 946–963, 5'-GAGAGAGCTCATAAAGACGCGCTAACACA-3'. The pAS2-1 ORF of CMV-As was amplified using specific primers (nt 120–138, 5'-GAGA- CCGGGGATGGCCTTTCCAAGTACC-3'; nt 941–959, 5'-GAGAGAGCTCATAAAGACGCGCTAACACA-3'). Amplified products were cloned into Smal/Sal-digested pACT2, which harbours the GAL4 activation domain (AD) (Clontech).

For the yeast three-hybrid assay, the ORFs encoding the 1a protein, 2a protein and MP were amplified using specific primers as described above and cloned into Smal/PstI-digested pBridge (Clontech). Alternative primer sets were used for ligation to NotI/BglII-digested pAS2-1, which encodes the GAL4 BD as follows. For 1a: nt 95–112, 5'-GAGAGCGGGGGCATGCGAGTCGTCCTGTTCC-3' and nt 3060–3076, 5'-GAGAGAGCTCATAAAGACGCGCTAACACA-3'. For 2a: nt 79–96, 5'-GAGAGCGGGGGCATGCGAGTCGTCCTGTTCC-3' and nt 2638–2655, 5'-GAGAGAGCTCATAAAGACGCGCTAACACA-3'. For the CP ORF: nt 124–141, 5'-GAGAGCGGGGGCATGCGAGTCGTCCTGTTCC-3' and nt 946–963, 5'-GAGAGAGCTCATAAAGACGCGCTAACACA-3'. Yeast colonies were transferred to Whatman no. 1 filter lift assay. Yeast colonies were transferred to Whatman no. 1 filter paper and filters were frozen in liquid nitrogen and allowed to thaw at room temperature. The filters were placed colonoy-side-up on a second filter paper pre-soaked in ‘Z’ buffer/X-Gal solution comprising 100 ml ‘Z’ buffer (60 mM Na2HPO4, 40 mM NaH2PO4, 10 mM KCl, 1 mM MgSO4, 1:2 ml X-Gal stock solution (20 mg 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside in N,N-dimethylformamide ml−1) and 0:27 ml β-mercaptoethanol). Filters were incubated at room temperature and interactions were detected by the appearance of blue colonies, generally after 5–60 min. Colonies isolated from the master plate were assayed quantitatively by the filter lift assay. Yeast colonies were transferred to Whatman no. 1 filter paper and filters were frozen in liquid nitrogen and allowed to thaw at room temperature. The filters were placed colonoy-side-up on a second filter paper pre-soaked in ‘Z’ buffer/X-Gal solution comprising 100 ml ‘Z’ buffer (60 mM NaH2PO4, 40 mM NaH2PO4, 10 mM KCl, 1 mM MgSO4, 1:2 ml X-Gal stock solution (20 mg 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside in N,N-dimethylformamide ml−1) and 0:27 ml β-mercaptoethanol). Filters were incubated at room temperature and interactions were detected by the appearance of blue colonies, generally after 5–60 min. Colonies isolated from the master plate were assayed quantitatively by the filter lift assay.

Yeast two-hybrid and three-hybrid analyses and quantification of β-galactosidase activity. Recombinant bait and prey vectors were transformed into Saccharomyces cerevisiae strain Y190, also provided with the MATCHMAKER GAL4 Two-hybrid System 2 (Clontech). Yeast was co-transformed by the lithium acetate method as described previously (Kim et al., 2002). Transformants were plated on solid synthetic dropout minimal complete medium containing 2% glucose and lacking leucine and tryptophan, or on medium lacking leucine, tryptophan and histidine, and for the yeast three-hybrid assay, on medium lacking methionine as well as these three amino acids. Transformants were spread on selective media and incubated at 30°C for 4–6 days. Protein interactions in transformants were detected by assaying colonies on selective media plates and confirmed by assessing β-galactosidase activity with a filter lift assay. Yeast colonies were transferred to Whatman no. 1 filter paper and filters were frozen in liquid nitrogen and allowed to thaw at room temperature. The filters were placed colonoy-side-up on a second filter paper pre-soaked in ‘Z’ buffer/X-Gal solution comprising 100 ml ‘Z’ buffer (60 mM NaH2PO4, 40 mM NaH2PO4, 10 mM KCl, 1 mM MgSO4, 1:2 ml X-Gal stock solution (20 mg 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside in N,N-dimethylformamide ml−1) and 0:27 ml β-mercaptoethanol). Filters were incubated at room temperature and interactions were detected by the appearance of blue colonies, generally after 5–60 min. Colonies isolated from the master plate were assayed quantitatively for β-galactosidase activity as described by O’Reilly et al. (1998). Genes encoding the SV40 large T antigen (pSV40) and the human p53 protein were inserted into pAS2-1 and pACT2, respectively, and used as positive interaction controls (Clontech). The human lamin C gene (pLaminC) was cloned downstream of the GAL4 AD in pACT2 and used as a non-interactive control (Clontech). One unit of β-galactosidase was defined as the amount that hydrolysed 1 μmol o-nitrophenyl β-D-galactopyranoside (ONPG) to o-nitrophenol and β-galactose min−1.
Co-immunoprecipitation of interacting proteins in yeast. Antibodies and co-immunoprecipitation analysis were carried out as described previously (Kim et al., 2002). Immune complexes were detected by Western blotting (Sambrook et al., 1989), using an anti-MP rabbit antibody and an anti-2a rabbit antibody. Briefly, yeast cells were resuspended with glass beads in lysis buffer (2 % Triton X-100, 1 % SDS, 100 mM NaCl, 10 mM Tris/HCl, pH 8-0, 1 mM EDTA) and vortexed five times for 5 s each at 1 min intervals. After centrifugation, the lysates were incubated with anti-2a or anti-MP antibody for 2 h at 4 °C. Immune complexes were collected and precipitated with 20 µl 50 % slurry of protein A-Sepharose beads (Bio-Rad). The beads were washed three times with PBS and the immunoprecipitates were separated by 8 % SDS-PAGE and transferred to a nitrocellulose membrane. Proteins were detected with anti-MP or anti-2a antibody and an alkaline phosphatase-conjugated secondary antibody (Bio-Rad).

RESULTS

Analysis of interactions among viral proteins detected by far-Western analysis

It was previously established that the 2a polymerase interacts with the 1a protein, resulting in the formation of an active replicase complex (O’Reilly et al., 1998; Kim et al., 2002). This finding was confirmed in this study, as shown in Figs 1 and 2. To determine whether the 2a polymerase protein interacted with other virally encoded proteins, interactions involving this protein were first assessed by far-Western analysis. ORFs encoding each viral protein were inserted downstream of the GST gene to obtain GST fusion proteins. The bacterially expressed proteins were purified by glutathione–agarose column chromatography, resolved by 8 % SDS-PAGE and transferred on to a nitrocellulose membrane, which was probed with the GST–2a protein. The 2a polymerase protein interacted with the 1a protein and the MP, but not with other virally encoded CMV proteins (Figs 1 and 2). As demonstrated in Fig. 1, the 2a polymerase protein interacted with GST–1a and GST–MP proteins (Fig. 1, lanes 1 and 4, respectively), while the other proteins did not interact with the GST–1a protein (Fig. 1, lanes 2, 3 and 5). Although a faint band in lane 5 was revealed by longer exposure, the CP did not interact with the 2a polymerase protein in the yeast two-hybrid assay (Fig. 2). These results showed that the 2a polymerase protein interacts not only with the 1a protein, but also with the MP.

Confirmation of 2a polymerase–MP interaction in the yeast two-hybrid system

To confirm the interaction between the 2a polymerase protein and the MP, constructs encoding each of the CMV-encoded proteins were cloned into vectors for yeast two-hybrid analysis. ORFs for 1a, 2a, 2b, MP and CP were inserted downstream of sequences encoding the GAL4 transcription factor BD, and the MP ORF was fused next to sequences encoding the GAL4 AD. These fusion plasmids were co-transformed into yeast, and colonies grown on a variety of selective media were analysed as described above. Only colonies co-transformed with plasmids containing BD–2a and AD–MP exhibited an intense blue colour after 30 min (Fig. 2). Colonies harbouring 2a polymerase constructs derived from CMV-As (lane 2) and CMV-Fny (lane 6) showed similar intensities. The CP did not interact with the 2a polymerase protein in the yeast two-hybrid assay (Fig. 2). These results showed that the 2a polymerase protein interacts not only with the 1a protein, but also with the MP.

![Fig. 1. Far-Western analysis of interactions involving the 2a polymerase protein as a probe. CMV-encoded proteins were expressed in E. coli, purified by glutathione–agarose chromatography and electrophoresed on an 8 % SDS-polyacrylamide gel. The proteins were transferred to a nitrocellulose membrane and allowed to interact with 32P-labelled 2a polymerase protein. Lanes: 1, 1a protein; 2, 2a polymerase protein; 3, 2b protein; 4, MP; 5, CP; 6, GST alone.](http://vir.sgmjournals.org)

![Fig. 2. Interaction of the MP with other virally encoded proteins.](http://vir.sgmjournals.org)
interact with the 1a protein, nor with itself (Fig. 2), suggesting that it does not form multimers in vivo.

In the filter-lift assays, yeast colonies containing the 2a polymerase and MP genes developed a blue colour within 30 min, whereas cells carrying p53 and pSV40 as positive controls took at least 2 h for colour to develop. Yeast cells harbouring the pLaminC and pSV40 constructs as negative controls did not undergo colour changes, even after overnight incubation. As shown in Fig. 3, a strong interaction between the 2a polymerase and MP was evident, compared with the control interaction between p53 and pSV40. β-Galactosidase activity was assayed by the ONPG method and transformants carrying the interacting 2a polymerase protein and MP constructs exhibited a 20- to 50-fold higher degree of activity than control cells carrying the 2a polymerase protein bait and a vector with the AD alone. Similar results were observed in bait–prey swapping experiments (data not shown). More than ten different colony variants assayed exhibited an activity approximately 20- to 50-fold higher than that of the control in three independent tests, suggesting that the 2a polymerase protein and the MP interact strongly in the yeast system.

A less intense but still significant colour change was detected as a result of the interaction of CMV-As MP with CMV-Fny 2a polymerase protein, compared with the blue colour generated by the cognate interaction between CMV-As MP and CMV-As 2a polymerase protein.

**Fig. 3.** Quantitative analysis of β-galactosidase specific activity of the 2a polymerase protein–MP interaction using ONPG. Specific activity was expressed as micromoles ONPG hydrolysed min⁻¹ (mg protein)⁻¹, from three independent experiments and from more than ten colonies in each experiment. Results are shown as means ± SD. Bars: 1, pAS2-1/2a only; 2, pAS2-1/2a and pACT2; 3, pAS2-1/2a and pACT2/MP; 4, p53 and pSV40; 5, pLaminC and pSV40.

**Fig. 4.** Co-immunoprecipitation analysis of the 2a polymerase–MP interaction. Total extracts from yeast cells producing 2a polymerase protein and MP were immunoprecipitated (IP) with anti-2a antibody and anti-MP antibody, as indicated, and fractionated by 8% SDS-PAGE. Proteins were transferred to a nitrocellulose membrane and immunoblotted (IB) with specific antibody. Lanes: 1, lysate from cells containing two empty vectors; 2, lysate from cells containing pAS2-1/2a and the vector pACT2 alone; 3, lysate from cells containing pAS2-1 and pACT2/MP; 4–6, lysates from cells from three independent colonies containing pAS2-1/2a and pACT2/MP; SM, prestained size marker (Invitrogen).

**Co-immunoprecipitation analysis confirming 2a polymerase–MP interaction**

To confirm the interaction between the 2a polymerase and MP observed by the yeast two-hybrid analysis, total yeast cell lysates were immunoprecipitated with anti-2a and anti-MP antibodies. The immune complexes were fractionated by SDS-PAGE and subjected to immunoblot analysis with anti-MP and anti-2a antibodies, respectively. Specific interactions were also identified in this experiment (Fig. 4). Total lysate from cells containing the vectors pAS2-1 and pACT2 as negative controls did not exhibit reactivity. As shown in Fig. 4 (lane 2), yeast cell lysates containing pAS2-1/2a and pACT2 were not recognized by specific antibodies. Yeast lysates containing pACT2 and pAS2-1/2a were also unreactive (Fig. 4, lane 3). Proteins in yeast cell lysates picked from three independent colonies containing pAS2-1/2a and pACT2/MP were detected by immunoblotting with the anti-2a antibody and anti-MP antibody, respectively, confirming a specific interaction between the 2a polymerase and the MP (Fig. 4, lanes 4–6).

**Detection of a 1a–2a–MP interaction by yeast three-hybrid analysis**

It was previously shown that the 2a polymerase protein interacts with the 1a protein in the formation of a replicase complex (Kim et al., 2002). Given the above results, it was of interest to test whether the MP was associated with the replicase complex or whether the MP competed with the 1a protein for interaction with the 2a protein. To determine whether the 1a–2a interaction interfered with the interaction between the 2a polymerase and MP, plasmid expressing...
the two proteins was introduced into yeast cells, including one in which the 2a polymerase gene was expressed from the vector pBridge, as described in Methods (Table 1). This system allows the detection of a third protein that can bridge the two other proteins, by disrupting or facilitating the interaction between the two. In this assay, the 1a protein, which does not interact directly with the MP, was found in a complex containing MP, but only when the 2a polymerase protein was expressed simultaneously (Table 1). Moreover, the MP and 1a protein also induced β-galactosidase activity (even more strongly) in the presence of the 2a polymerase protein, suggesting that the 2a polymerase protein connects the 1a protein to MP (Table 1). Changing the baits and preys yielded the same results. The MP neither inhibited nor modified the strong interaction between the 1a protein and the 2a polymerase protein (Table 1).

### DISCUSSION

The interaction between the 1a protein and 2a polymerase protein observed here was not particularly surprising, since replicase activity co-purifies with both proteins (Hayes & Buck, 1990) and the yeast two-hybrid assay has been used to detect specific interactions between the 1a and 2a polymerase proteins of BMV and CMV (O’Reilly et al., 1998). The 2a polymerase protein has been shown to interact with the helicase domain of the 1a protein (O’Reilly et al., 1998). The 2a polymerase protein has been shown to interact with the helicase domain of the 1a protein (O’Reilly et al., 1998). The 2a polymerase protein has been shown to interact with the helicase domain of the 1a protein (O’Reilly et al., 1998). The 2a polymerase protein has been shown to interact with the helicase domain of the 1a protein (O’Reilly et al., 1998). The 2a polymerase protein has been shown to interact with the helicase domain of the 1a protein (O’Reilly et al., 1998). The 2a polymerase protein has been shown to interact with the helicase domain of the 1a protein (O’Reilly et al., 1998). The 2a polymerase protein has been shown to interact with the helicase domain of the 1a protein (O’Reilly et al., 1998).

Although the temporal order of the interactions in vivo is not yet known, it is suspected that the replicase and MP cooperate in regulating the intercellular movement of progeny viral RNA by an unknown mechanism. CMV transfers its genomes across plasmodesmata in the form of a ribonucleoprotein complex. Although co-localization of the MP and the replicase complex in CMV has not been detected, it is assumed that the MP can locate adjacent to the site of RNA synthesis. This would result in a more coordinated recognition of newly synthesized RNA by the MP and more rapid transfer of viral RNA to and through the plasmodesmata. During infection by TMV, despite the system being quite different from CMV, virus replication complexes containing membrane-associated replicase, genomic RNAs and MP move to adjacent cells through plasmodesmata as large bodies (Asurmendi et al., 2004; Kawakami et al., 2004). It has been proposed that these complexes contain all the necessary components that initiate rapid spread of infection (Kawakami et al., 2004).

<table>
<thead>
<tr>
<th>Bait</th>
<th>Prey</th>
<th>Third protein</th>
<th>LacZ expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>MP</td>
<td>2a</td>
<td>+++</td>
</tr>
<tr>
<td>1a</td>
<td>MP</td>
<td>Empty</td>
<td>−</td>
</tr>
<tr>
<td>MP</td>
<td>1a</td>
<td>2a</td>
<td>+ + +</td>
</tr>
<tr>
<td>MP</td>
<td>1a</td>
<td>Empty</td>
<td>−</td>
</tr>
<tr>
<td>2a</td>
<td>1a</td>
<td>MP</td>
<td>+ + +</td>
</tr>
<tr>
<td>2a</td>
<td>1a</td>
<td>Empty</td>
<td>+ + +</td>
</tr>
<tr>
<td>1a</td>
<td>2a</td>
<td>MP</td>
<td>+ + +</td>
</tr>
<tr>
<td>2a</td>
<td>1a</td>
<td>Empty</td>
<td>+ + +</td>
</tr>
</tbody>
</table>

A previous study with CMV has implied a role for the polymerase protein in virus movement. It was shown that two nucleotide changes in the polymerase gene affected the elicitation of a hypersensitive response and restriction of CMV to a local lesion in cowpea (Kim & Palukaitis, 1997). Previous studies have demonstrated that the CMV 2a polymerase protein can be detected as early as 3 h post-infection in squash and tobacco protoplasts (Gal-On et al., 1994; Kim & Palukaitis, 1997), although phosphorylated 2a polymerase protein was undetectable prior to 48 h post-infection in the tobacco protoplast system (Kim et al., 2002). Phosphorylated 2a polymerase protein then continued to accumulate up to 72 h post-infection. Phosphorylated 2a polymerase protein did not interact with the 1a protein, but the continued accumulation of the 2a polymerase protein suggested that it has other functions. Thus, it is conceivable that the 2a polymerase is involved in viral movement. In addition to this, some data from previous studies with TMV have shown the possibility of cooperation between the replicase and MP. It was shown that the failure of cell-to-cell movement was not caused by the MP. It is also possible that the replicase and MP associate with viral RNA and/or with a host factor(s) and collaborate indirectly (Hirashima & Watanabe, 2001, 2003).
Interaction of CMV-Fny 2a polymerase protein with CMV-As MP in our yeast two-hybrid system suggests that subgroup IA CMV-Fny MP, which exhibits 94.3% amino acid sequence identity with subgroup IB CMV-As MP, may have a similar conformation to that of subgroup IB MPs. The 2a polymerase proteins of CMV-Fny and CMV-As also share 93.8% amino acid sequence identity.

Co-expression of CMV MP and CP complements defects in the movement of Tomato mosaic virus and Potato virus X, but this effect is not seen with either protein alone (Tamai et al., 2003). CMV MP requires its cognate CP in order to transfer viral genomes through the plasmodesmata (Canto et al., 1997; Nagano et al., 2001). Truncated CMV MP lacking the 33 C-terminal amino acids supports the cell-to-cell movement of CMV as well as the chimeric BMV (Nagano et al., 1997). It is suggested that it is the C-terminal 33 amino acids of CMV MP that are involved in conferring specificity for the viral genome. A recent report showed that the C-terminal 33 amino acids of CMV MP affected virus movement, RNA binding and inhibition of infection and translation (Kim et al., 2004). Deletion of the C-terminal 33 amino acids resulted in CP-independent cell-to-cell movement, although CP was still required for long-distance movement. In addition, the truncated MP had a greater affinity for the viral RNA than the wild-type MP. The biological differences may be a consequence of differences in the architecture of binding of the two MPs (Andreev et al., 2004). It has been suggested that the CP may alter the conformation of the MP available for complex formation (Kim et al., 2004). The CP also may not interact directly with the MP, but via a host protein(s), which may explain the failure to detect direct interactions between the MP and CP as shown here.

In conclusion, our study constitutes the first report showing that, among CMV virally encoded proteins, the MP interacts specifically with the 2a polymerase protein. Further studies should allow us to identify the function of the interaction between the 2a polymerase and MP.

ACKNOWLEDGEMENTS

This work was supported by the BK21 program, a grant from the Korea Science & Engineering Foundation (KOSEF) (R01-2000-00143) and a grant from the Plant Signalling Network Research Center (R11-2003-008-02001-0).

REFERENCES


