

Interaction of the movement and coat proteins of *Maize streak virus*: implications for the transport of viral DNA

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We have shown previously that the movement protein (MP) and coat protein (CP) of *Maize streak virus* (MSV) are both required for systemic infection. Towards understanding the roles of these two proteins in virus movement, each was expressed in *E. coli* and interactions of the MP with viral DNA or CP were investigated using south-western, gel overlay and immunoprecipitation assays. Unlike the CP, the MP did not bind to viral DNA but it interacted with the CP *in vitro* and an MP–CP complex was detected in extracts from MSV-infected maize, indicating the potential for an interaction *in vivo*. Microinjection showed that the MP could prevent the nuclear transport of an MSV CP–DNA complex in maize and tobacco cells. These results are consistent with a model in which the MP diverts a CP–DNA complex from the nucleus (where viral DNA replication takes place) to the cell periphery, and in co-operation with the CP, mediates the cell-to-cell movement of the viral DNA. In this respect, the MSV MP and CP have functional analogy with the BC1 and BV1 proteins, respectively, of the *Begomovirus* genus of the *Geminiviridae*.

Introduction

Maize streak virus (MSV) is a species of the genus *Mastrevirus* of the family *Geminiviridae* (Briddon & Markham, 1995; Stanley *et al.*, 1999). It has a single-stranded (ss) circular DNA genome (Howell, 1984; Lazarowitz, 1988; Mullineaux *et al.*, 1984) and is transmitted by the leafhopper *Cicadulina mbila*. Neither MSV nor MSV DNA can be mechanically transmitted but cloned DNA is infectious when inoculated by *Agrobacterium*-mediated DNA transfer (agroinoculation; Boulton *et al.*, 1989a; Grimsley *et al.*, 1987). MSV, like other geminiviruses, replicates in the nucleus (Davies *et al.*, 1987; Goodman, 1981; Nagar *et al.*, 1995) via a double-stranded (ds) DNA intermediate, which is bidirectionally transcribed (Morris-Krsinich *et al.*, 1985; Wright *et al.*, 1997). Of the four viral genes, *REP* and *REP A*, derived from the open reading frames (ORFs) *C1* and *C2* on the complementary (C) sense strand, are required for virus replication and probably transactivation of

virion-sense gene expression, and *MP* and *CP*, formerly ORFs *V1* and *V2* on the virion (V) sense strand, are required for systemic infection (Boulton *et al.*, 1989b, 1993; Lazarowitz *et al.*, 1989). Gene *MP* encodes a 10.9 kDa (101 amino acids) protein (Mullineaux *et al.*, 1988). Mutagenesis studies have shown that *MP* encodes a movement protein (MP) which is required for cell-to-cell movement of virus (Boulton *et al.*, 1993). Gene *CP* encodes coat protein (CP) (Morris-Krsinich *et al.*, 1985) which is required for systemic movement, encapsidation of MSV DNA into virions and insect transmission (Boulton *et al.*, 1989b; Lazarowitz *et al.*, 1989).

The mechanism of movement of the bipartite geminiviruses of the genus *Begomovirus* has been investigated intensively. Two proteins encoded by the B DNA component are involved in movement, BV1 (BR1) and BC1 (BL1) (Lazarowitz, 1992). The BC1 protein of *Bean dwarf mosaic virus* (BDMV) increases the size exclusion limit (SEL) of plasmodesmata of cells into which it is injected, and the protein mediates viral DNA transport from cell to cell (Noueir *et al.*, 1994; Rojas *et al.*, 1998) and is thus regarded as the viral movement protein. In contrast, the BC 1 protein of *Squash leaf curl virus* (SqLCV) does not bind DNA but is present in virus-induced tubules that cross the walls of meristematic phloem tissues (Ward *et al.*, 1997). The BV1 protein is nuclear localized, binds to viral DNA

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(Noueir *et al.*, 1994; Pascal *et al.*, 1994) and acts as a nuclear shuttle protein. Interaction of the two proteins regulates the directionality of intracellular viral DNA transport (Sanderfoot *et al.*, 1996). The CP of the bipartite viruses is dispensable for systemic infection in an adapted host (Gardiner *et al.*, 1988; Pooma *et al.*, 1996), although it can aid the movement or pathogenicity of such viruses (Qin *et al.*, 1998). In contrast, the CP of mastreviruses is required for plant infection (Boulton *et al.*, 1989b, 1993; Liu *et al.*, 1998; Woolston *et al.*, 1989). The *E. coli*-expressed CPs of MSV (Liu *et al.*, 1997) and the monopartite begomovirus *Tomato yellow leaf curl virus* (TYLCV) interact with viral DNA and localize in the nucleus in insect and plant cells (Kunik *et al.*, 1998; Liu *et al.*, 1997; 1999; Palanichelvam *et al.*, 1998). Furthermore, microinjection of MSV CP with fluorescently labelled viral DNA into plant cells has demonstrated that the MSV CP can facilitate transport of viral DNA into the nucleus, suggesting that the CPs of the monopartite viruses may perform at least some of the functions of BV1 (Liu *et al.*, 1999). Although the CP of SqLCV has also been shown to bind ssDNA and localize to the nucleus, these functions are also provided by the BV1 protein (Qin *et al.*, 1998), and thus CP-mediated nuclear targeting may be required only in initially infected cells following insect transmission of begomovirus particles.

The MPs of mastreviruses are required for cell-to-cell movement (Boulton *et al.*, 1993; Liu *et al.*, 1998); all have a stretch of hydrophobic amino acids that are predicted to form a transmembrane structure (Boulton *et al.*, 1993). The MSV MP is associated with plasmodesmata in infected tissue (Dickinson *et al.*, 1996), and an MSV MP–GFP fusion translocates from cell to cell when expressed in epidermal cells of maize leaves (Kotlitzky *et al.*, 2000). However, the precise role of MSV MP in virus movement is still unknown. Likely, there is functional analogy to the BC1 of the begomoviruses. Towards understanding the MSV MP functions and MSV movement mechanism, the DNA- and CP-binding capacity of *E. coli*-expressed MP was analysed using south-western, gel overlay and immunoprecipitation assays. The effect of MSV MP on CP-mediated viral DNA transport was investigated by microinjection. The results showed that MP, unlike CP, was unable to bind viral DNA, but can interact with CP. Furthermore, the MP prevented, or diminished, nuclear accumulation of an MSV CP–DNA complex in microinjected plant cells. A model for the intracellular transport of MSV DNA is suggested.

Methods

■ **Expression of the MSV MP and CP genes in *E. coli*.** The MSV MP gene was amplified by PCR with the infectious clone (pMSV-Ns; Boulton *et al.*, 1991) as template. An *Nde*I site (underlined) and six histidine codons were introduced into the forward primer (5' GGTCACATATCATATGGATCCGCATCACCATCATCACCATGATCC ACAGAACGCC 3') and the 3' end primer (5' GACATGGCTAGATCTTATCCCG 3') contained a *Bgl*II site. For expression of the His-tagged MSV MP (6 × His-MP) in *E. coli*, the *Nde*I/*Bgl*II-digested PCR

product was ligated into *Nde*I/*Bam*HI-linearized pET3a to produce pETMP. The MP gene and the linkages were sequenced to confirm the integrity of the insert. The MSV 6 × His-MP was produced in *E. coli* using the T7 RNA polymerase overexpression system (Studier & Moffatt, 1986). For this, pETMP was transfected into *E. coli* strain BL21(DE3) and 6 × His-MP expression was induced as described previously for the MSV CP (Liu *et al.*, 1997). Immunoblotting (Towbin *et al.*, 1979) with anti-MSV MP serum (a gift from C. Woolston, Dept of Applied Biology, University of Hull, UK) or Ni–NTA conjugate (Qiagen) was used to identify the 6 × His-MP. The protein was purified from *E. coli* cells (centrifuged from a 100 ml culture) using Ni–NTA resin (Qiagen) and native or denaturing conditions (8 M urea) as described in the 'QIAexpressionist' (Qiagen) except that the non-denaturing lysis buffer contained 1 mM lysozyme and 1 mM PMSF. In all cases, suspensions were sonicated three times (30 s each with a 1 min interval) prior to stirring at room temperature. The relative efficiency of MP extraction by the two lysis procedures was examined by SDS–PAGE of supernatants ('cleared lysates') after resuspension of an aliquot in Laemmli loading buffer and boiling for 10 min. For elution of 6 × His-MP under denaturing conditions, the elution buffer contained 6 M guanidine hydrochloride and 0.2 M acetic acid. Denatured MP was refolded by immediately dialysing the eluate in 1 l PBS pH 7.5 twice at 4 °C, each for 1.5 h. For raising anti-MSV MP serum, the column-purified 6 × His-MP was further purified by SDS–PAGE through a 15% preparative gel (Bio-Rad). The Coomassie blue-stained MP band was recovered from the gel using a membrane cap (cut-off 3.5 kDa) and electroelution (Electro-eluter, Bio-Rad) for 36 h at 4 °C. Prior to injection, the MP was dialysed twice against 1.5 l PBS pH 7.4 at 4 °C, each for 1.5 h. The concentration of MP was determined using the DC protein assay kit (Bio-Rad) based on the Bradford assay.

Expression and purification of MSV CP were carried out as described (Liu *et al.*, 1999). Where appropriate, the *E. coli*-expressed CP and MP were compared with native protein (in the form of total protein extracts of MSV-infected maize) by SDS–PAGE and immunoblotting.

■ **Preparation of anti-MSV MP serum.** Anti-MSV MP serum was raised by intramuscular injection into a rabbit as described (Pinner & Markham, 1990) except that 100 µg of purified 6 × His-MP was used for each immunization. Serum was recovered from blood collected from the rabbit 10 days after the third boost and tested against 6 × His-MP and infected plant extracts by immunoblotting. This antiserum was used for south-western and gel overlay experiments.

■ **South-western analysis of MP–DNA binding.** To assess MSV MP–DNA binding, both purified 6 × His-MP and total *E. coli* cell extracts were used for south-western analysis as described by Sukegawa & Blobel (1993). All samples and controls were prepared, fractionated by SDS–PAGE, and transferred to membrane as described previously for CP extracts (Liu *et al.*, 1997). The [α -³²P]dCTP-labelled MSV and pUC probes were prepared as before (Liu *et al.*, 1997) and added to reaction buffer containing 100 or 250 mM KCl for binding assays.

■ **Gel overlay assays.** To investigate MSV MP–CP interaction *in vitro*, the gel overlay assay was used following the method described by Schwank *et al.* (1995) except that MP or CP was visualized using immunochemical staining. Cells expressing CP (collected from 100 ml of culture) were sonicated in 20 ml of lysis buffer (10 mM Tris–HCl pH 8.0, 100 mM NaCl, 1 mM EDTA, 10% glycerol, 1 mM DTT, 1 mM PMSF). For cells expressing MP, 0.1% Triton X-100 was added to solubilize the MP. A total of 20 µl of each cell extract was fractionated by SDS–PAGE on a 15% separating gel and electrotransferred onto a nitrocellulose membrane. After renaturation of the proteins, the membrane on which CP extracts were bound was overlaid with 20 ml of total extract of cells expressing 6 × His-MP from pETMP, whereas the membrane onto which

MP extracts were bound was overlaid with the extract containing CP. After washing the membranes, immunoblotting with anti-MP or anti-CP serum, respectively, was used to determine whether the proteins used in the overlay buffer were bound to immobilized proteins and therefore retained on the membrane.

■ **Immunoprecipitation assay.** To determine whether the MP and CP could interact in infected plants, immunoprecipitation assays were used. About 2 g of MSV-infected maize leaf was ground to a fine powder in liquid nitrogen and the total proteins were extracted by grinding for another 2 min in 2 ml HEGK–MND buffer pH 7.5 (Blanar & Rutter, 1992) lacking $MgCl_2$ but containing 5 mM EDTA. The supernatant was collected by centrifugation at 13 000 r.p.m. for 30 min at 4 °C. The MSV MP or CP present in the supernatant was immunoprecipitated using anti-MSV MP or CP serum as described by Blanar & Rutter (1992) except that the CP or MP was subsequently visualized using immunochemical staining. Immunoblotting with anti-MSV CP serum was used to identify CP in the sample precipitated with anti-MP serum, while anti-MSV MP serum was used to determine whether MP co-precipitated with the CP. Anti-NADP-dependent malic enzyme (NADP-ME) serum (a gift from Jane Langdale, Dept of Plant Sciences, University of Oxford, UK) was used to determine whether proteins abundant in maize tissue were present in the precipitate.

■ **Microinjection.** Microinjection of maize and tobacco leaf cells and labelling of MSV DNA with TOTO-1 dye (Molecular Probes) was carried out as described previously (Liu *et al.*, 1999). $6 \times$ His-MP was co-injected into the cells with MSV TOTO-1-ss or -dsDNA to assess its ability to mediate cell-to-cell transport of DNA. The effect of the MSV $6 \times$ His-MP on the localization of CP DNA in the cells was investigated by injecting TOTO-1-DNA, CP and MP together in a ratio of 1:1:1 into the cells. The CP (0.5 mg/ml) was first mixed with the TOTO-1-DNA for 15 min, and then the MP (0.5 mg/ml) was added. The mixture was injected into the cells with an equal volume of Texas Red dextran. The effect of $6 \times$ His-MP at 1 mg/ml was also tested. An MP-nonexpressing extract of pET3a-transformed *E. coli* cells was used, in place of the $6 \times$ His-MP, for the control injections.

Results

Expression and purification of MSV MP

The construct pETMP, which carries the MSV MP gene sequence with six histidine codons linked to its 5' end, was used to express MSV MP in *E. coli* BL21(DE3) cells. Comparison of the Coomassie-stained protein profiles of cleared *E. coli* lysates prepared under native conditions did not reveal an additional band of the predicted molecular mass (approx. 11.5 kDa) from cells transformed with pETMP compared to those with pET3a [Fig. 1A, compare lanes E-MP(LN) and E-O]. However, when lysis was done under denaturing conditions, a protein with an apparent molecular mass of 14 kDa was seen in extracts of cells transformed with pETMP [Fig. 1A, lane E-MP(LD)]. This protein was detected by immunoblotting using anti-MSV MP serum (Fig. 1B) or Ni-NTA conjugate (Fig. 1C), confirming that it was $6 \times$ His-MP. The protein was visible from 6 h post-induction (p.i.) and reached a maximum level at 20 h p.i. (data not shown). As expected, because of the addition of six histidine residues to its N terminus, the *E. coli*-expressed MP had a slightly higher

molecular mass than the MSV MP present in infected plant extracts [compare lanes E-MP(LD) and P-MP; Fig. 1B]. Several bands were recognized by both anti-MSV MP serum and Ni-NTA conjugate in *E. coli* extracts transformed with pETMP; these were of the sizes expected from multimeric forms of the $6 \times$ His-MP [arrowed in Fig. 1B, C, lanes E-MP(LD)]. The eluate obtained following Ni-NTA purification, under denaturing conditions, of extracts of the $6 \times$ His-MP-expressing cells, was subjected to SDS-PAGE. Coomassie blue staining showed that the majority of the protein co-migrated with mono- and multimeric forms of $6 \times$ His-MP (Fig. 1D, MP-COL) although contaminating proteins could also be seen. Thus further purification was necessary to isolate $6 \times$ His-MP for use in antiserum production and this was done by gel elution of the 14 kDa band (arrowed in Fig. 1D). When the purity of this protein was assessed by PAGE, $6 \times$ His-MP multimers were again detected (arrowed in Fig. 1D, E, lanes MP-GEL). About 2 mg of MP was obtained from 1 l of *E. coli* culture via this method.

MSV MP does not bind to viral DNA *in vitro*

Many plant virus MPs are known to bind to viral nucleic acids (Deom *et al.*, 1992; McLean *et al.*, 1993; Pascal *et al.*, 1994). To determine whether the MSV MP binds DNA, the $6 \times$ His-MP was used in south-western assays. Neither the column-purified MP nor the MP in cell lysates bound to MSV ss or dsDNA even when incubation was done in the presence of low (100 mM) KCl concentration [Fig. 2A, C, lanes MP-COL and E-MP(LD)]. In contrast, the MSV CP bound both ds and ssDNA (Fig. 2, lanes CP-PUR). Immunoblot analysis of the membranes showed that the MP was retained after the south-western assay (Fig. 2B, D), indicating that the lack of DNA binding was not because of the loss of MP.

MSV MP interacts with MSV CP *in vitro*

As MSV CP binds to both ss and dsDNA [as described above, and in Liu *et al.* (1997)] and mediates the nuclear transport of viral DNA (Liu *et al.*, 1999) it is possible that the MP interacts with the CP to facilitate virus movement. To investigate this, gel overlay assays were carried out using the *E. coli*-expressed $6 \times$ His-MP and CP and binding was assessed using their respective antisera. When MP was blotted on the membrane, and MSV CP was used as the overlay, anti-CP serum recognized the CP controls and a single band in the MP extract lane (Fig. 3A), which migrated to the same position in the gel as did the $6 \times$ His-MP [approx. 14 kDa, compare with Fig. 3B, lane E-MP(LD)]. Conversely, when CP was blotted on the membrane and the $6 \times$ His-MP was used as the overlay, the anti-MP serum recognized the MP control and a major band of approximately 28 kDa (Fig. 3B). This latter band co-migrated with *E. coli*-expressed CP, and CP extracted from virus particles [Fig. 3A, lanes E-CP(LN) and V-CP]. In numerous experiments,

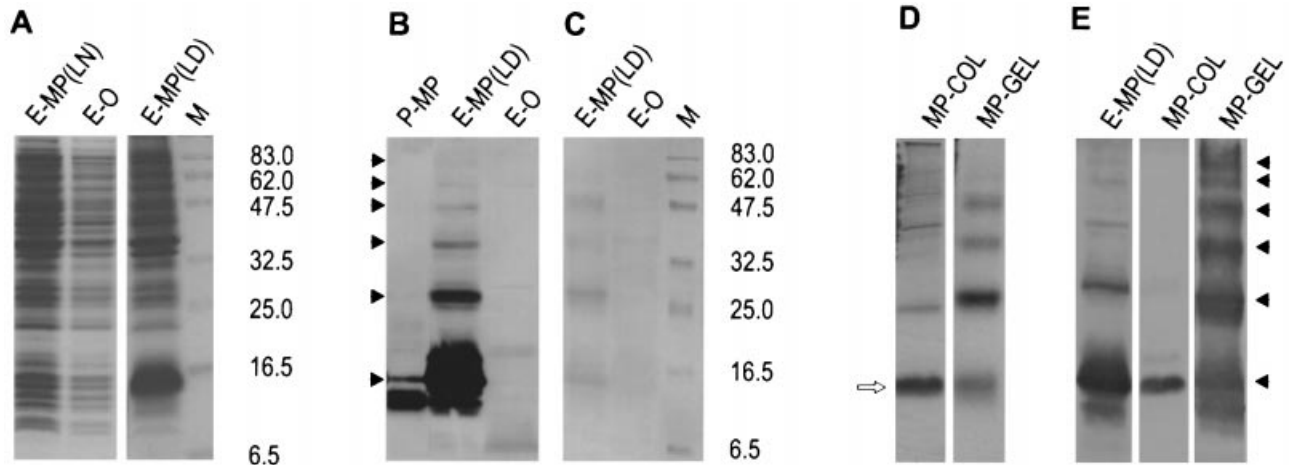


Fig. 1. Gel electrophoretic analysis of MSV MP expressed in *E. coli* transformed with pETMP. (A) Coomassie blue staining of proteins present in the supernatant fraction following lysis of *E. coli* under native [E-MP(LN)] or denaturing [E-MP(LD)] conditions. (B)–(C) Immunoblot analysis of proteins in the denatured lysate supernatant [E-MP(LD)] with anti-MSV MP serum (B) or with nickel conjugate (C). P-MP denotes MSV-infected plant extract, in which can be seen the native MP of 10.9 kDa in abundance and a thin band equivalent to about 14 kDa above it, presumed to be overspill from the adjacent track [6 × His-MP in E-MP(LD)]. Arrows show the position of monomeric and multimeric forms of the MP. (D) Coomassie blue staining of 6 × His-MP purified using an Ni-NTA column (MP-COL) and the protein(s) obtained (lane MP-GEL) following gel elution of the MP-specific band (open arrow). (E) Immunoblots obtained with anti-MSV MP serum. Arrows indicate the monomeric and multimeric forms of MP. E-O denotes lysate of *E. coli* transformed with pET3a. Positions of the molecular size markers (M) are indicated.

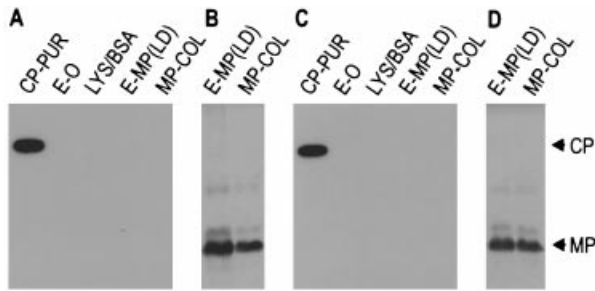


Fig. 2. ‘South-western’ assay of DNA-binding ability of MSV MP. Denatured lysates of *E. coli* cells transformed with pETMP (MP-LD) or purified 6 × His-MP (MP-COL) were separated by PAGE and tested for their ability to bind MSV dsDNA (A) and ssDNA at 100 mM KCl concentration (C) by south-western analysis. MSV CP, purified from *E. coli* (CP-PUR), was used as a positive control for DNA binding. Lysates of *E. coli* cells transformed with pET3a (E-O) and lysozyme and bovine serum albumin (LYS/BSA) were used as negative controls. After autoradiography, immunoblotting with anti-MSV MP serum was used to confirm the presence of MP on the membranes (B and C). Positions of the viral proteins (MP and CP) are indicated.

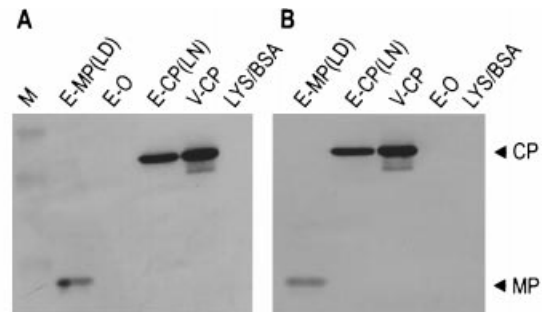


Fig. 3. Interaction of MSV MP with CP analysed by gel overlay assay. Extracts of *E. coli* cells expressing MSV 6 × His-MP (E-MP) or CP (E-CP) and purified MSV (V-CP) were separated by PAGE and overlaid with purified MSV CP (A) or 6 × His-MP (B). Membranes were then subjected to immunoblotting with either anti-MSV CP serum (A) or anti-MP serum (B). Control proteins [lysozyme (LYS) and bovine serum albumin (BSA)] and extracts of *E. coli* transformed with pET3a (E-O) were used to determine the specificity of protein–protein interactions. Positions of the viral proteins and molecular size markers (M) are indicated. LD and LN, as for Fig. 1.

in the absence of overlay, we have never observed cross-reactions between anti-CP serum and MP or anti-MP serum and CP (and see also Fig. 4). No bands were detected in lanes containing lysozyme and BSA or *E. coli* proteins. Thus, the data suggest a specific CP–MP interaction. In a second experiment, using a different batch of protein extracts, a specific interaction was again seen, but in this case, the relative amounts of staining obtained with the CP overlay and anti-CP serum were greater than that seen with the MP overlay and antiserum. It was not

possible in these experiments to assess the molar ratio of MP:CP.

An MP–CP complex can be immunoprecipitated from MSV-infected plant extracts

Immunoprecipitation was used to determine whether MSV MP could interact with CP in infected plant extracts. When anti-MP serum was used to produce the precipitate, immuno-

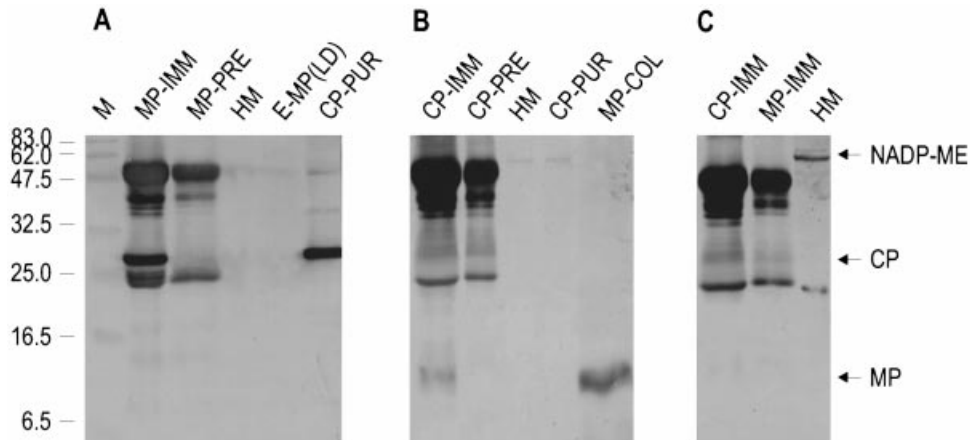


Fig. 4. Interaction of MSV MP with CP in MSV-infected maize cell extracts identified by immunoprecipitation assay. Cell extracts were treated with anti-MSV MP serum (MP-IMM, panel A) or anti-CP serum (CP-IMM, panel B) and the precipitates were analysed by immunoblotting of the PAGE-separated proteins using anti-MSV CP serum (A) or anti-MSV MP serum (B). Pre-immune serum was used as a control for each precipitation (MP-PRE and CP-PRE). Maize anti-NADP malic enzyme (NADP-ME) serum was used to search for the presence of contaminating host protein in the immunoprecipitates (C). An uninfected maize extract (HM) and purified CP and MP were used as controls for the immunoblot. E-MP(LD), CP-PUR, MP-COL are as described in Figs 2 and 3. Positions of the viral proteins, NADP-ME and molecular size markers (M) are indicated.

blotting of the precipitated proteins with anti-CP serum revealed the presence of CP (Fig. 4A, compare lane MP-IMM with lane CP-PUR) and when anti-CP serum was used for immunoprecipitation, MP was also present in the precipitate (Fig. 4B, compare lane CP-IMM with MP-COL). No CP- or MP-specific bands were seen when preimmune sera were used for immunoprecipitation (Fig. 4A, B, lanes MP-PRE and CP-PRE). The additional bands visible in the lanes containing precipitate are immunoglobulins present in the antibody-antigen complex. NADP-ME was not detected in either precipitate (Fig. 4C): thus the presence of both MSV MP and CP in the precipitates likely reflects a specific interaction between the two viral proteins, rather than contamination of the precipitate by proteins abundant in the maize extract.

MSV MP affects CP-mediated nuclear transport of DNA in tobacco and maize cells

Although interaction between the MP and CP have been shown *in vitro* (Fig. 3) and in plant extracts (Fig. 4), the data do not show that an MP-CP complex can be produced in living cells, nor do they suggest a function for the complex. We have previously suggested (Liu *et al.*, 1999; Kotlitzky *et al.*, 2000) that the MP-CP interaction could be important for regulating the directionality of DNA (or a CP-DNA complex) transport, for example by directing it from the nucleus to the cell periphery. Microinjection studies were used, therefore, to determine whether the $6 \times$ His-MP could affect nuclear accumulation of MSV DNA in the presence of MSV CP. We have previously shown (Liu *et al.*, 1999) that the MSV CP can facilitate nuclear transport of TOTO-1-labelled MSV DNA in both maize and tobacco cells, with nuclear fluorescence being clearly visible within 5 min and being maintained for at least

15 min after injection. However, in the current study when MP was present in the injection mixture (at both concentrations tested), negligible nuclear fluorescence was seen in the cells injected with TOTO-1-labelled ds (Fig. 5A-D) or ssDNA (Fig. 5I-L), even at 15 min after injection. This inhibition of nuclear accumulation of MSV DNA was not caused by the presence of contaminating *E. coli* proteins because when purified extracts of *E. coli* transformed with pET3a were used in place of the $6 \times$ His-MP, the nuclei in the injected cell showed clear fluorescence (Fig. 5E-H, M-P). Microinjection of the MP and TOTO-1-DNA alone did not result in nuclear accumulation of fluorescence (not shown). The relatively high level of autofluorescence in the injected cells made it impossible to determine the site to which the labelled DNA was redirected when the MP was present in the injection mixture. The results of the microinjections are summarized in Table 1. Fluorescence was not observed in other neighbouring cells after injection of higher levels of DNA or longer incubation times. Indeed, fluorescence usually began to fade about 30 min after injection (not shown).

Discussion

MSV, like other geminiviruses, replicates via a dsDNA intermediate that also acts as template for bidirectional transcription (Morris-Krsinich *et al.*, 1985; Wright *et al.*, 1997). Its MP and CP, encoded by the virion-sense transcripts, are required for systemic infection and pathogenicity (Boulton *et al.*, 1989b, 1993; Lazarowitz *et al.*, 1989). MSV particles or MSV DNA, presumably in a nucleoprotein complex, must move from cell to cell and long distance to establish a systemic infection, as shown with other plant viruses (Carrington *et al.*, 1996; Gilbertson & Lucas, 1996) and must also enter the

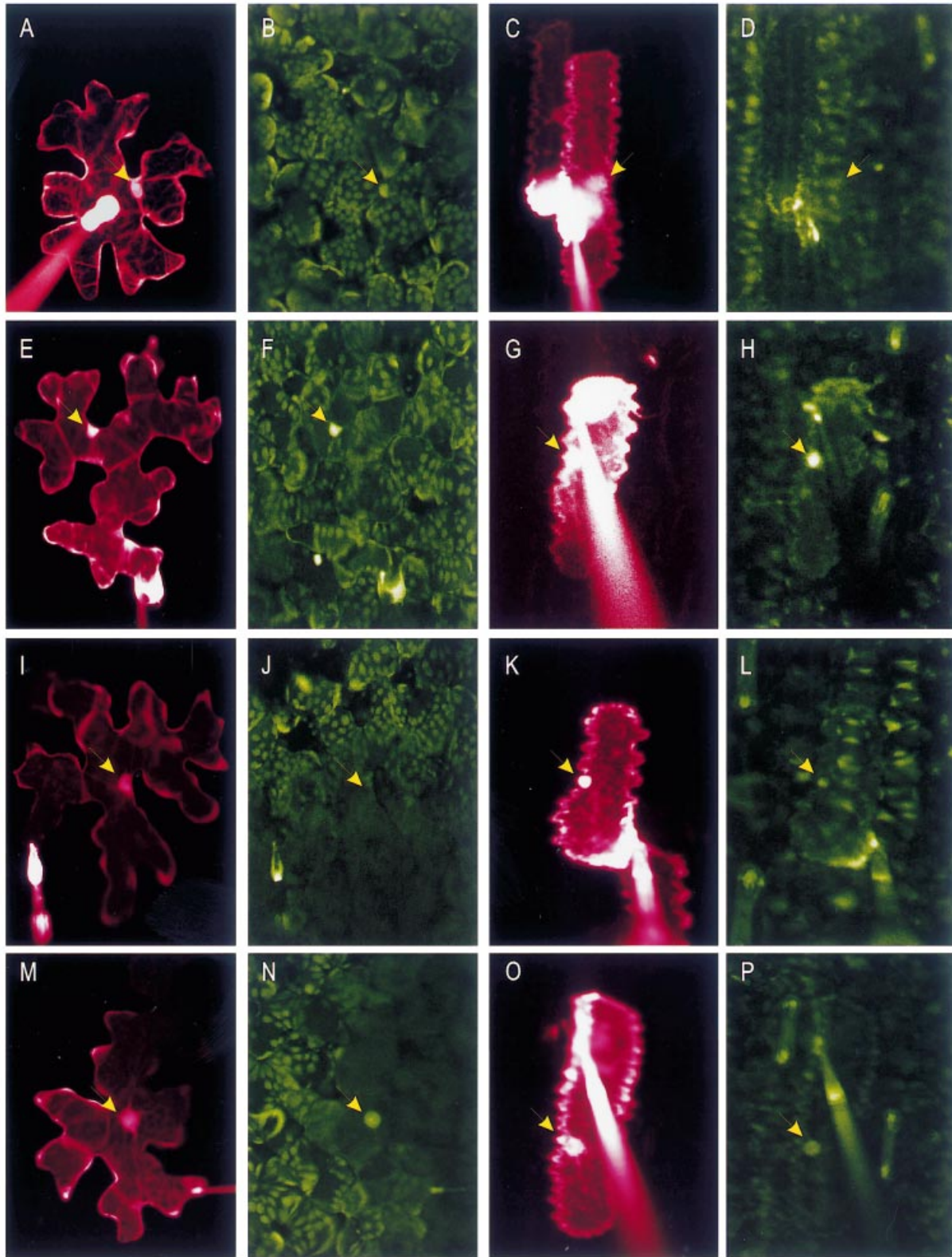


Fig. 5. Localization of TOTO-1-DNA-CP in tobacco (A, B, E, F, I, J, M, N) and maize (C, D, G, H, K, L, O, P) epidermal cells in the presence (A, B, C, D, I, J, K, L) or absence (E, F, G, H, M, N, O, P) of $6 \times$ His-MP. Cells shown in (A)–(H) were injected with TOTO-1-dsDNA, those in (I)–(P) with ssDNA. All images were taken 15 min after injection. Images (A), (C), (E), (G), (I), (K), (M) and (O) were obtained with an excitation wavelength of 568 nm to visualize the Texas Red dextran and the images of the

Table 1. Effect of MSV MP on nuclear transport of MSV TOTO-1-DNA-CP

Plant cells	Sample injected*	No. of injected cells†	No. of cells with nuclear localization of TOTO-1-DNA	Time of nuclear fluorescence (min)‡
Tobacco	ssDNA	5	0	0§
	dsDNA	5	0	0
	CP/ssDNA	5	5	5
	CP/dsDNA	5	5	5
	CP/ssDNA/MP	5	0	0
	CP/dsDNA/MP	5	1	15
	CP/ssDNA/EP	5	5	5
	CP/dsDNA/EP	5	5	5
	Maize	CP/ssDNA	5	5
CP/dsDNA		5	5	5
CP/ssDNA/MP		5	1	15
CP/dsDNA/MP		5	0	0
CP/ssDNA/EP		5	5	5

* DNA was labelled with TOTO-1. CP, Coat protein; EP, *E. coli* protein; MP, movement protein. CP was pre-incubated with single-stranded (ss) or double-stranded (ds) MSV TOTO-1-DNA prior to co-injection with MP.

† Successful injections.

‡ Earliest time at which fluorescence (TOTO-1-DNA) was seen in the nucleus.

§ When no nuclear fluorescence was seen after 5 min, observations were repeated 15 min after injection. At this point, the time of nuclear fluorescence was rated as '0'.

|| Weak nuclear fluorescence was seen 15 min after injection.

nucleus for replication and virus assembly. Mutational analysis showed that both the MSV MP and the CP are required for cell-to-cell and long-distance movement (Boulton *et al.*, 1989*b*, 1993). MSV CP was shown to bind ss and dsDNA (Liu *et al.*, 1997), it localized in the nucleus in insect cells and tobacco protoplasts and mediated the nuclear transport of viral DNA in maize or tobacco cells (Liu *et al.*, 1999). Unlike the bipartite geminiviruses which encode two MPs, including a nuclear localization (or shuttle) protein, BV1, and a protein responsible for targeting a BV1-DNA complex to the cell periphery (BC1) (Lazarowitz, 1992; Sanderfoot & Lazarowitz, 1995), MSV encodes only one MP (Boulton *et al.*, 1989*b*, 1993; Lazarowitz *et al.*, 1989; Mullineaux *et al.*, 1988) and the CP functions as the nuclear localization protein. Although MSV MP is thought to be associated with plasmodesmata (Dickinson *et al.*, 1996), and has been shown to move from cell to cell as an MP-GFP fusion (Kotlitzky *et al.*, 2000), the role of MSV MP in the cell-to-cell movement of virus or viral DNA is unclear. In this study, we expressed 6 × His-MP in *E. coli* cells and it, like many other plant virus proteins expressed in *E. coli* (Gamer *et al.*,

1992; Garcia *et al.*, 1989*a, b*; Maia & Bernardi, 1996; Noueir *et al.*, 1994; Pascal *et al.*, 1994), appears to be functional. Purification of the 6 × His-MP proved to be difficult, as most of the expressed protein was insoluble; only a small amount could be obtained under non-denaturing conditions although a larger amount of the MP was obtained under denaturing conditions. The expressed MP possibly localized in the membranes [it is predicted to contain a transmembrane domain (Boulton *et al.*, 1993)], but could be released with 0.1% Triton X-100. Purification of MSV MP was carried out under denaturing conditions, yet interestingly, the purified MP formed multimers even in SDS-PAGE. MSV MP has a leucine-rich domain that may be important for protein-protein interactions, and although the functional form of MSV MP in infected plants is not known, it is possible that MSV MP forms multimers to mediate virus movement.

Many plant virus MPs have been shown to bind viral nucleic acid and interact with plasmodesmata (reviewed in Carrington *et al.*, 1996). However, the His-tagged MSV MP did not bind viral DNA (Fig. 2). This result suggests that MSV

TOTO-1-DNA (B, D, F, H, J, L, N, P) were obtained using an excitation wavelength of 488 nm. Arrows indicate the position of the nucleus in the injected cell. In most images at 568 nm, the position of the injection needle containing fluorescent inoculum can be seen, and in panels (C) and (K) there is evidence that inoculum was injected into two maize cells.

MP might be functionally different from the majority of plant virus MPs, although an association MSV MP with plasmodesmata of infected plants has been observed (Dickinson *et al.*, 1996). As MSV CP interacts with DNA and mediates the nuclear transport of viral DNA (Liu *et al.*, 1997, 1999), it is possible that MSV MP co-operates with the CP to move the viral DNA from the infected cell nucleus to the periphery of the cell, thereby enabling cell-to-cell movement through the plasmodesmata. Such interaction of the MP with the CP is indicated by the gel overlay assays (Fig. 3) and the MP-CP complex immunoprecipitated from MSV-infected plant cells (Fig. 4). It is thus proposed that the MSV MP moves a CP-DNA complex from cell to cell. Since no MSV MP has been detected associated with purified MSV particles (Mullineaux *et al.*, 1988), it is likely that the movement is accomplished in the form of a nucleoprotein complex. However, the structure of the MP-CP-DNA complex and the regulation of its formation is unknown.

The CP-mediated nuclear transport of MSV DNA in maize and tobacco cells was disrupted in the presence of the $6 \times$ His-MP (Fig. 5). We therefore propose that the MP requires the assistance of the CP, which binds to viral DNA (Liu *et al.*, 1997), to facilitate MSV movement towards the cell periphery and we cannot rule out the possibility that MP facilitates nuclear export of the CP or CP-DNA. Clearly, there is some analogy with begomovirus nuclear shuttling and cell-to-cell movement functions. The limited protein-coding capacity of geminivirus monopartite genomes requires proteins to have multiple functions compared to those encoded by the bipartite begomoviruses. Indeed, it has been shown that the begomovirus BV1 and CP proteins exhibit functional redundancy; the SqLCV CP localizes to the nucleus and masks BV1 mutants (Qin *et al.*, 1998). The evolutionary relationship of these proteins is underlined by their amino acid similarity (Kikuno *et al.*, 1984). The close relationship between mastrevirus and begomovirus CPs has also been reported (Mullineaux *et al.*, 1985).

The MSV MP has many of the functions of the begomovirus BC1 protein, but the means by which the MSV complex (MP-CP-DNA) moves from cell to cell is not yet clear. For example, it is not certain whether the MP binds at plasmodesmata to enable CP-DNA to move through and if it moves with the complex or remains at the binding site. Dickinson *et al.* (1996) reported that MSV MP is associated with the secondary plasmodesmata in MSV-infected tissue, but no stable association was detected by Kotlitzky *et al.* (2000) using an MSV MP-GFP fusion, although in this case no other viral proteins were present in the cell. It is also not clear how the directionality of the movement is regulated. Nuclear targeting of the MSV CP is mediated by a nuclear localization signal located at the N terminus of the protein (Liu *et al.*, 1997, 1999). It is possible that this signal is blocked by the presence of the MP, although other modifications of the CP (such as a change in phosphorylation state) cannot be ruled out. We propose a

model in which CP moves DNA into the nucleus, but CP-MP interaction redirects MSV DNA to the cell periphery for movement to a neighbouring cell.

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