



LUND UNIVERSITY

Dysfunctionality of a tobacco mosaic virus movement protein mutant mimicking threonine 104 phosphorylation.

Karger, E M; Frolova, O Yu; Fedorova, N V; Baratova, L A; Ovchinnikova, T V; Susi, P; Makinen, K; Rönstrand, Lars; Dorokhov, Yu L; Atabekov, J G

Published in:
Journal of General Virology

DOI:
[10.1099/vir.0.18972-0](https://doi.org/10.1099/vir.0.18972-0)

2003

[Link to publication](#)

Citation for published version (APA):

Karger, E. M., Frolova, O. Y., Fedorova, N. V., Baratova, L. A., Ovchinnikova, T. V., Susi, P., Makinen, K., Rönstrand, L., Dorokhov, Y. L., & Atabekov, J. G. (2003). Dysfunctionality of a tobacco mosaic virus movement protein mutant mimicking threonine 104 phosphorylation. *Journal of General Virology*, 84(Pt 3), 727-32. <https://doi.org/10.1099/vir.0.18972-0>

Total number of authors:
10

General rights

Unless other specific re-use rights are stated the following general rights apply:
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

Read more about Creative commons licenses: <https://creativecommons.org/licenses/>

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

LUND UNIVERSITY

PO Box 117
221 00 Lund
+46 46-222 00 00

Short
CommunicationDysfunctionality of a tobacco mosaic virus
movement protein mutant mimicking threonine 104
phosphorylationE. M. Karger,¹ O. Yu. Frolova,¹ N. V. Fedorova,¹ L. A. Baratova,¹
T. V. Ovchinnikova,² P. Susi,³ K. Makinen,⁴ L. Ronnstrand,^{5†}
Yu. L. Dorokhov¹ and J. G. Atabekov¹

Correspondence

Joseph Atabekov
atabekov@genebee.msu.su¹Department of Virology and A. N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, Vorobiovy Gory Moscow 119899, Russia²M. M. Shemyakin and Yu. A. Ovchinnikov Institut of Bioorganic Chemistry, Moscow, Russia³Joint Biotechnology Laboratory, Biocity, Turku, Finland⁴University of Helsinki, Institute of Biotechnology, Biocenter, Helsinki, Finland⁵Ludwig Institute of Cancer Research, Biomedical Center, Uppsala, Sweden

Replication of tobacco mosaic virus (TMV) is connected with endoplasmic reticulum (ER)-associated membranes at early stages of infection. This study reports that TMV movement protein (MP)-specific protein kinases (PKs) associated with the ER of tobacco were capable of phosphorylating Thr¹⁰⁴ in TMV MP. The MP-specific PKs with apparent molecular masses of about 45–50 kDa and 38 kDa were revealed by gel PK assays. Two types of mutations were introduced in TMV MP gene of wild-type TMV U1 genome to substitute Thr¹⁰⁴ by neutral Ala or by negatively charged Asp. Mutation of Thr¹⁰⁴ to Ala did not affect the size of necrotic lesions induced by the mutant virus in *Nicotiana tabacum* Xanthi nc. plants. Conversely, mutation of Thr to Asp mimicking Thr¹⁰⁴ phosphorylation strongly inhibited cell-to-cell movement. The possible role of Thr¹⁰⁴ phosphorylation in TMV MP function is discussed.

Received 12 November 2002

Accepted 10 December 2002

Cell-to-cell movement of tobacco mosaic virus (TMV) is mediated by a 30 kDa movement protein (MP) encoded by TMV RNA (reviewed by Carrington *et al.*, 1996; Lazarowitz & Beachy, 1999; Tzfira *et al.*, 2000). It has been shown that P30 accumulates in plasmodesmata of TMV-infected and MP-transgenic plants (Tomenius *et al.*, 1987; Atkins *et al.*, 1991; Oparka *et al.*, 1997; Heinlein *et al.*, 1998) to increase their permeability (Wolf *et al.*, 1991; Ding *et al.*, 1992). The TMV MP co-aligns with microtubules (McLean *et al.*, 1995; Heinlein *et al.*, 1995) and is tightly associated with ER-derived membranes of infected cells (Reichel & Beachy, 1998; Heinlein *et al.*, 1998). Furthermore, it has been reported that ER-enriched fractions from infected tobacco leaves contain TMV MP, RNA and replicase, implying that virus replication and protein synthesis take place in this compartment (Mas & Beachy, 1999). Importantly, it is known that P30 accumulates in the cell wall (CW) fraction of transgenic plants as a phosphoprotein (Citovsky *et al.*, 1993; Waigmann *et al.*, 2000) and that the CW-associated

protein kinase(s) (PKs) can use MP as substrate. C-proximal residues Ser²⁵⁸, Thr²⁶¹ and Ser²⁶⁵ have been identified as phosphorylation sites *in vitro* (Citovsky *et al.*, 1993) and *in vivo* (Waigmann *et al.*, 2000). TMV encoding a mutant MP mimicking phosphorylation of these sites by negatively charged Asp substitution is unable to move from cell to cell in *Nicotiana tabacum* plants (Waigmann *et al.*, 2000). It has been proposed that C-terminal phosphorylation of TMV MP abolishes its ability to promote virus spread (Waigmann *et al.*, 2000).

Watanabe *et al.* (1992) reported that C-terminal residues 234–261 are required for TMV MP phosphorylation in tobacco protoplasts, although it is clear that in TMV-infected protoplasts MP can be phosphorylated at multiple internal phosphorylation sites (Haley *et al.*, 1995). In particular, two distinct domains (residues 61–114 and 212–231) can be substrates for PK(s) other than the CW-associated PK(s) mentioned above. Similarly, it has been shown that Ser³⁷ and Ser²³⁸ of tomato mosaic tobamovirus (ToMV) MP can be phosphorylated in protoplasts. The presence of Ser at position 37 or phosphorylation of Ser³⁷ is important for ToMV MP functionality (Kawakami *et al.*, 1999). Thus, the results from different groups of workers

†Present address: Division of Experimental Clinical Chemistry, Lund University, Malmo University Hospital, Malmo, Sweden.

Published ahead of print on 20 December 2002 as DOI 10.1099/vir.0.18972-0.

imply that TMV MP is phosphorylated *in vivo*, although it is hard to say in what cellular compartments MP phosphorylation occurs. It is important to emphasize that only the C-proximal sites of CW-associated TMV MP are found phosphorylated *in planta* (Waigmann *et al.*, 2000), while multiple internal sites of MP could be phosphorylated in infected protoplasts (Haley *et al.*, 1995; Kawakami *et al.*, 1999). Despite the apparent contradiction, the various results are not necessarily incompatible. Thus, TMV MP might be transiently phosphorylated when subjected to processes of phosphorylation/dephosphorylation at its internal sites by cytoplasmic PK(s), whereas only the C-proximal sites are selectively phosphorylated by CW-associated PKs.

The TMV genome is accepted widely to be translocated from cell to cell as an MP-RNA complex. Moreover, it has been reported that TMV MP is an efficient repressor of *in vitro* translation and phosphorylation of MP prevents its translation-repressing ability (Karpova *et al.*, 1999). Possible roles of viral MP phosphorylation in regulation of TMV genome expression have been discussed recently by Lee & Lucas (2001).

Microsomal fractions from leaves of *N. tabacum* var. Samsun were isolated by sucrose gradient centrifugation, as described by Mas & Beachy (1999), and analysed for PK activity using preparations of bacterially expressed TMV U1 (His)₆-MP as a substrate for labelling in the

presence of [γ -³²P]ATP. Purification of (His)₆-MP was carried out as described by Karpova *et al.* (1997). The level of MP phosphorylation activity varied along the sucrose gradient with maximums in fractions 2–4 and 12–15 (Fig. 1a). The presence of endoplasmic reticulum (ER) luminal-binding protein (BiP), an ER membrane resident protein (Reichel & Beachy, 1998), was revealed in fractions 2 and 11–13 by Western blotting (Fig. 1b). These data indicate that MP-specific PK activity was at a maximum in ER-containing fractions of the sucrose density gradient (Fig. 1a, b). However, there was no BiP in fractions 4 and 15 that phosphorylate the MP. Thus, the PK(s) that phosphorylate the MP may not reside exclusively in the ER.

The influence of different divalent metal cations on the MP-specific ER-associated PK activity was examined in a series of experiments. Mg²⁺ and particularly, Mn²⁺ were found to stimulate MP phosphorylation, whereas no stimulation was detected in the presence of Ca²⁺ (Fig. 1c). There was no PK activity when no metal cation was added to the reaction. The number and molecular masses of the MP-specific PKs in ER-associated fractions were determined by gel PK assays (Zhang & Klessig, 1997). The recombinant TMV MP and myelin basic protein (MBP), a universal substrate for mitogene-activated PKs, were compared using the assay. MP or MBP were copolymerized with acrylamide and 20 μ l of each fraction was loaded on a gel. Two or three major bands, with a

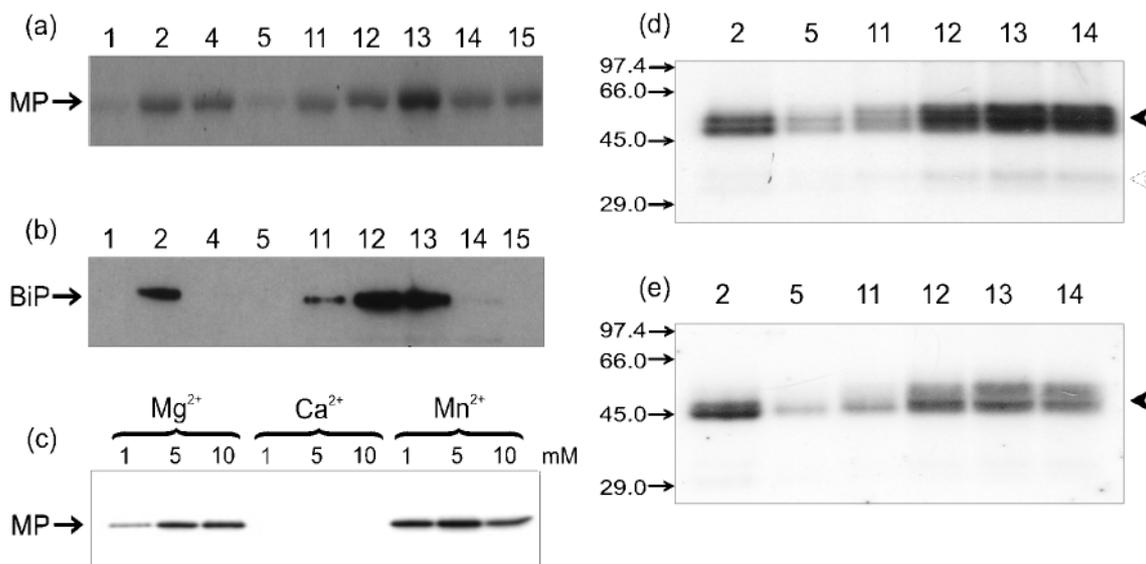


Fig. 1. Characterization of MP-specific ER-associated PK activity. (a) PAGE of TMV MP phosphorylated *in vitro* by ER-enriched sucrose gradient fractions. Identification of the MP band was performed in a series of separate Western blot assays. (b) Western blot analysis of BiP in the same fractions. Lane numbers correspond to the number of the sucrose gradient fraction. (c) Electrophoresis of TMV MP phosphorylated *in vitro* by a sucrose gradient fraction with high PK activity in the presence of divalent cations. Cation concentrations (mM) are indicated over the lanes. (d, e) Gel PK assays with recombinant TMV MP (d) and MBP (e) as substrates. Lane numbers correspond to sucrose density gradient fractions. Positions of TMV MP (a, c), BiP (b) and standard marker proteins (in kDa) (d) are indicated. The closed arrowhead indicates the position of kinases larger than 45 kDa; the open arrowhead denotes a minor component of 38 kDa.

molecular mass somewhat higher than 45 kDa, were revealed by this approach in ER-associated fractions when TMV MP (Fig. 1d) or MBP (Fig. 1e) was used as a substrate. It is possible that the multiple bands revealed in the ER-containing fractions by gel PK assay represent different isoforms or degradation products of MP/MBP-specific PK(s). The similarity of molecular masses (45–50 kDa) of the ER-associated MP-specific PKs described above and of mitogen-activated protein kinases Ntf4 and Ntf6 (Wilson *et al.*, 1995) might reflect a relationship. Remarkably, the activity of PKs described in this study and that of Ntf4 and Ntf6 PKs was stimulated by Mn²⁺ and Mg²⁺. A lower molecular mass minor component (molecular mass of 38 kDa) was revealed as a minor band in the gel PK assays (Fig. 1d, open arrowhead). Matsushita *et al.* (2000) report that the cytoplasmic 38 kDa plant casein kinase II (CKII) is capable of phosphorylating ToMV MP and it is possible that the minor 38 kDa component detected by gel PK assay (Fig. 1d) represents CKII. Radioactive bands were not observed when the control samples (no exogenous protein added as a substrate) were analysed.

To localize the sites phosphorylated by the ER-associated PK activity in TMV MP, (His)₆-MP was phosphorylated *in vitro* in the presence of a microsomal fraction, [γ -³²P] ATP and 1 mM MnCl₂. After additional purification of phosphorylated protein on Ni-NTA resin in the presence of 6 M guanidium/HCl, pH 8.0 (Qiagen), according to the manufacturer's protocol, and immobilization on thiopropyl-Sepharose 6B (Sigma), the MP was digested by sequence-grade trypsin (Sigma) and the resulting peptides were analysed by two different approaches. First, two-dimensional peptide mapping was applied to separate the phosphopeptides (Fig. 2a). Analyses were performed on HTLE-7002 equipment in accordance with the manufacturer's protocol (CBS Scientific). Several ³²P-labelled tryptic peptides could be seen in the phosphopeptide map (Fig. 2a), which is consistent with TMV MP being phosphorylated by ER-associated PK at multiple sites. The five most prominent spots (Fig. 2a, numbers 1–5), corresponding presumably to major ³²P-labelled tryptic peptides, were subjected to phospho-amino acid analysis; it was demonstrated that ³²P was incorporated into spots corresponding to phosphoserine in peptides 2–5 and to phosphothreonine in peptide 1 (data not shown). Second, the ³²P-labelled phosphopeptides were separated by HPLC and isolated in sufficient amounts for partial amino acid sequencing (first five amino acids were analysed in each peptide). In the present work, we have focused on characterization of peptide 1, which was found to contain Thr¹⁰⁴ in the N-terminal sequence ADEAT. Indirect evidence for the importance of Thr¹⁰⁴ in TMV MP activity was provided by analyses of functional reversions of Thr¹⁰⁴ dysfunctional mutants (Deom & He, 1997; Boyko *et al.*, 2002). It is also noteworthy that Thr¹⁰⁴ is conserved in MPs encoded by different ToMV (Koonin *et al.*, 1991).

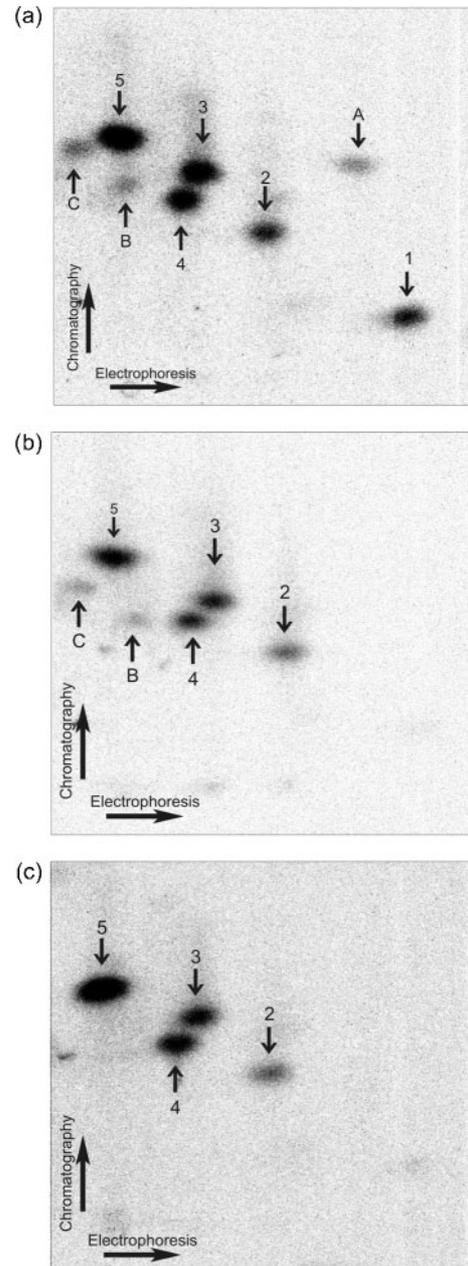


Fig. 2. Two-dimensional mapping of ³²P-labelled tryptic peptides of wild-type and mutant TMV MPs phosphorylated by ER-associated PK activity. (a) Wild-type MP, (b) T¹⁰⁴A and (c) T¹⁰⁴D mutant MPs. The five most prominent spots are indicated by numbers and the remaining minor spots by letters. The directions of electrophoresis and chromatography are indicated by arrows at the bottom left-hand corner.

To study the importance of Thr¹⁰⁴ for function, point mutations were introduced into the recombinant (His)₆-MP gene to replace Thr¹⁰⁴ in bacterially expressed MPs with (i) alanine, which prevents phosphorylation, or (ii) aspartate, which is believed to mimic protein phosphorylation (Waigmann *et al.*, 2000). The two mutant MP forms obtained were designated as T¹⁰⁴A and T¹⁰⁴D, respectively.

To examine the phosphorylation patterns of mutated MPs, the preparations of bacterially expressed recombinant T¹⁰⁴A and T¹⁰⁴D proteins were phosphorylated as described

above and analysed by two-dimensional peptide mapping. Fig. 2(b, c) show that substitution of Thr¹⁰⁴ by either of the amino acids Ala or Asp led to the disappearance of

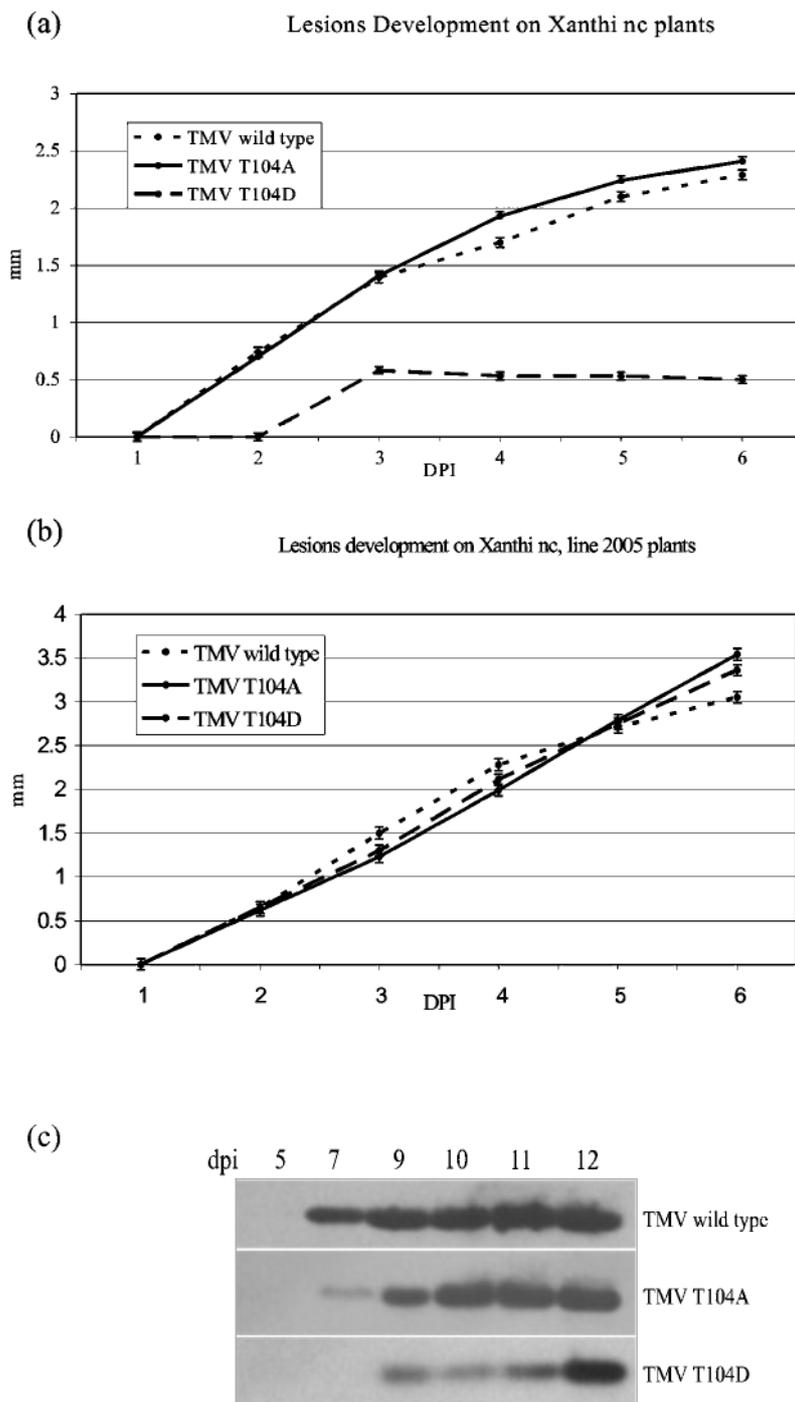


Fig. 3. Dysfunctionality of TMV MP caused by substitution of Thr¹⁰⁴ by Asp (T¹⁰⁴D TMV) is complemented in MP-transgenic plants. Development of local lesions induced by wild-type, T¹⁰⁴D and T¹⁰⁴A TMV MP mutants on leaves of (a) *N. tabacum* cv. Xanthi nc. and (b) MP-transgenic *N. tabacum* cv. Xanthi nc., line 2005, plants. Mean values (with SE bars) of local lesions diameter (mm) calculated for not less than 50 local lesions are presented. (c) Western blot analysis of TMV coat protein from the upper leaves of *N. tabacum* var. Samsun plants infected with wild-type TMV and MP mutants T¹⁰⁴D and T¹⁰⁴A. Days post-inoculation (DPI) are indicated above the lanes.

one major and one minor spot (Fig. 2a, labelled 1 and A) from the phosphopeptide map. The major spots, 2–5 (Fig. 2), were still present in the phosphopeptide maps of the mutant MPs. These results indicate that (i) Thr¹⁰⁴ can be phosphorylated *in vitro* by ER-associated PK(s) in wild-type MP and (ii) the level of phosphorylation of phosphopeptides 2–5 was not decreased by replacement of Thr¹⁰⁴ with either of the amino acids. It is noteworthy that two minor spots (Fig. 2a, b, labelled B and C) observed in phosphopeptide maps of wild-type and T¹⁰⁴A MP were missing from phosphorylated T¹⁰⁴D MP (Fig. 2c). One can speculate that substitution of Thr¹⁰⁴ by Asp may change the MP conformation so that these two sites are not exposed to phosphorylation. Finally, the ER-associated PKs responsible for T¹⁰⁴A, T¹⁰⁴D and wild-type MP phosphorylation were examined by gel PK assay. The number and apparent molecular masses of PKs revealed were similar in the experiments when wild-type and mutant MPs were used as substrate (data not shown). Therefore, no particular ER-associated PK was responsible for the Thr¹⁰⁴ phosphorylation only.

To elucidate the functional importance of Thr¹⁰⁴ for TMV cell-to-cell movement, mutations were introduced into the MP gene of a full-length TMV U1 cDNA copy to substitute Thr¹⁰⁴ by Ala or Asp in modified MP. The mutant viruses referred to as TMV T¹⁰⁴A and TMV T¹⁰⁴D, respectively, were compared by inoculation of indicator plants reacting to TMV infection by production of local lesions (*N. tabacum* cv. Xanthi nc.) or systemic symptoms (*N. tabacum* var. Samsun, *N. benthamiana*). Opposite halves of the same leaf were inoculated and mean values for at least 10 inoculated leaves were compared. The specific infectivity levels (number of the local lesions produced by 1.5 µg RNA on Xanthi nc. leaves) of wild-type and T¹⁰⁴A transcripts were very similar, as was the size of lesions produced by T¹⁰⁴A and wild-type RNA (Fig. 3a). In contrast, the specific infectivity of T¹⁰⁴D RNA transcripts dramatically decreased (13 ± 5 and 86 ± 17 lesions per half-leaf were induced by T¹⁰⁴D and wild-type TMV, respectively). It should be emphasized that only tiny local lesions were produced by T¹⁰⁴D mutant (Fig. 3a), suggesting that the Thr to Asp substitution at position 104 strongly inhibited virus cell-to-cell movement. However, our results do not rule out that the MP produced by mutant T¹⁰⁴D is less stable than wild-type and T¹⁰⁴A MPs.

It is important to note that the difference in development of local lesions induced by the wild-type TMV and T¹⁰⁴D mutant was abolished when Xanthi nc. line 2005 plants transgenic for TMV MP gene were inoculated (Fig. 3b). Therefore, the movement deficiency of T¹⁰⁴D MP could be complemented *in trans* by MP produced in transgenic plants. In addition, wild-type and T¹⁰⁴A TMV induced a severe mosaic on *N. tabacum* var. Samsun, whereas TMV T¹⁰⁴D mutant caused only a mild mosaic on tobacco plants. Fig. 3(c) shows that accumulation of TMV T¹⁰⁴D in upper systemically infected leaves of *N. tabacum* var.

Samsun plants was clearly delayed, whereas the time-course of accumulation of TMV T¹⁰⁴A and wild-type TMV in upper leaves was similar. In order to test the stability of the T¹⁰⁴A and T¹⁰⁴D mutations, the progeny of the mutant viruses was isolated from *N. benthamiana* plants. No reversions were detected by sequencing cDNA of T¹⁰⁴A and T¹⁰⁴D MP genes obtained by RT-PCR. No symptom differences could be detected on *N. tabacum* var. Samsun and Xanthi nc. plants inoculated with primary RNA transcripts or with the progeny of mutant viruses.

In conclusion, our *in vivo* experiments have shown that: (i) replacement of Thr¹⁰⁴ in TMV MP with neutral Ala did not cause significant changes in cell-to-cell movement of TMV, indicating that phosphorylation of Thr¹⁰⁴ was not essential for MP functions; (ii) substitution of Thr¹⁰⁴ by a negatively charged Asp residue led to a strong inhibition of the local lesion development in Xanthi nc. tobacco. This inhibition could be eliminated in Xanthi nc. plants transgenic for MP gene. Presuming that this substitution functionally mimics phosphorylation, we suggest that Thr¹⁰⁴ phosphorylation renders TMV MP dysfunctional. If this is the case, it seems logical to hypothesize that Thr¹⁰⁴ phosphorylation *in vivo* represents a defence mechanism that protects the plant from virus infections. It should be mentioned that inactivation of the MP by the Asp¹⁰⁴ mutation may not be directly due to mimicry of phosphorylation but due to the change of the MP conformation. It is evident that our data do not provide direct evidence that MP is in fact phosphorylated at Thr¹⁰⁴ during infection. Alternatively, it may be phosphorylated only transiently *in vivo*. Experiments on examination of *in vivo* Thr¹⁰⁴ phosphorylation are in progress.

ACKNOWLEDGEMENTS

The authors thank R. N. Beachy for generously providing the seeds of Xanthi nc. line 2005 plants, K. Lehto for providing viral full-length cDNA copy TMV 304 and V. K. Novikov for purified virus preparations. This work was supported by Fogarty International Collaboration Awards (FIRCA, grant PAR-99-008). E.K. was supported in part by The Centre for International Mobility (grant no. HAO214). P. S. was supported by Academy of Finland (grant nos 48719 and 53864).

REFERENCES

- Atkins, D., Hull, R., Wells, B., Roberts, K., Moore, P. & Beachy, R. N. (1991). The tobacco mosaic virus 30K movement protein in transgenic tobacco plants is localized to plasmodesmata. *J Gen Virol* 72, 209–211.
- Boyko, V., Ashby, J. A., Suslova, E., Ferralli, J., Sterthaus, O., Deom, C. M. & Heinlein, M. (2002). Intramolecular complementing mutations in tobacco mosaic virus movement protein confirm a role for microtubule association in viral RNA transport. *J Virol* 76, 3974–3980.
- Carrington, J. C., Kasschau, K. D., Mahajan, S. K. & Schaad, M. C. (1996). Cell-to-cell and long-distance transport of viruses in plants. *Plant Cell* 8, 1669–1681.

- Citovsky, V., McLean, B. G., Zupan, J. R. & Zambryski, P. (1993).** Phosphorylation of tobacco mosaic virus cell-to-cell movement protein by a developmentally regulated plant cell wall-associated protein kinase. *Genes Dev* **7**, 904–910.
- Deom, C. M. & He, X. Z. (1997).** Second-site reversion of a dysfunctional mutation in a conserved region of the tobacco mosaic tobamovirus movement protein. *Virology* **232**, 13–18.
- Ding, B., Haudenshield, J. S., Hull, R. J., Wolf, S., Beachy, R. N. & Lucas, W. J. (1992).** Secondary plasmodesmata are specific sites of localization of the tobacco mosaic virus movement protein in transgenic tobacco plants. *Plant Cell* **4**, 915–928.
- Haley, A., Hunter, T., Kiberstis, P. & Zimmern, D. (1995).** Multiple serine phosphorylation sites on the 30 kDa TMV cell-to-cell movement protein synthesized in tobacco protoplasts. *Plant J* **8**, 715–724.
- Heinlein, M., Epel, B. L., Padgett, H. S. & Beachy, R. N. (1995).** Interaction of tobamovirus movement proteins with the plant cytoskeleton. *Science* **270**, 1983–1985.
- Heinlein, M., Padgett, H. S., Gens, J. S., Pickard, B. G., Casper, S. J., Epel, B. L. & Beachy, R. N. (1998).** Changing patterns of localization of the tobacco mosaic virus movement protein and replicase to the endoplasmic reticulum and microtubules during infection. *Plant Cell* **10**, 1107–1120.
- Karpova, O. V., Ivanov, K. I., Rodionova, N. P., Dorokhov, Yu. L. & Atabekov, J. G. (1997).** Nontranslatability and dissimilar behavior in plants and protoplasts of viral RNA and movement protein complexes formed *in vitro*. *Virology* **230**, 11–21.
- Karpova, O. V., Rodionova, N. P., Ivanov, K. I., Kozlovsky, S. V., Dorokhov, Y. L. & Atabekov, J. G. (1999).** Phosphorylation of tobacco mosaic virus movement protein abolishes its translation repressing ability. *Virology* **261**, 20–24.
- Kawakami, S., Padgett, H. S., Hosokawa, D., Okada, Y., Beachy, R. N. & Watanabe, Y. (1999).** Phosphorylation and/or presence of serine 37 in the movement protein of tomato mosaic tobamovirus is essential for intracellular localization and stability *in vivo*. *J Virol* **73**, 6831–6840.
- Koonin, E. V., Mushegian, A. R., Ryabov, E. V. & Dolja, V. V. (1991).** Diverse groups of plant RNA and DNA viruses share related movement proteins that may possess chaperone-like activity. *J Gen Virol* **72**, 2895–2903.
- Lazarowitz, S. G. & Beachy, R. N. (1999).** Viral movement proteins as probes for intracellular and intercellular trafficking in plants. *Plant Cell* **11**, 535–548.
- Lee, J. Y. & Lucas, W. J. (2001).** Phosphorylation of viral movement proteins: regulation of cell-to-cell trafficking. *Trends Microbiol* **9**, 5–8.
- Mas, P. & Beachy, R. N. (1999).** Replication of tobacco mosaic virus on endoplasmic reticulum and role of the cytoskeleton and virus movement protein in intracellular distribution of viral RNA. *J Cell Biol* **147**, 945–958.
- Matsushita, Y., Hanazawa, K., Yoshioka, K., Oguchi, T., Kawakami, S., Watanabe, Y., Nishiguchi, M. & Nyunoya, H. (2000).** *In vitro* phosphorylation of the movement protein of tomato mosaic tobamovirus by a cellular kinase. *J Gen Virol* **81**, 2095–2102.
- McLean, B. G., Zupan, J. & Zambryski, P. C. (1995).** Tobacco mosaic virus movement protein associates with the cytoskeleton in tobacco cells. *Plant Cell* **7**, 2101–2114.
- Oparka, K. J., Prior, D. A., Santa Cruz, S., Padgett, H. S. & Beachy, R. N. (1997).** Gating of epidermal plasmodesmata is restricted to the leading edge of expanding infection sites of tobacco mosaic virus (TMV). *Plant J* **12**, 781–789.
- Reichel, C. & Beachy, R. N. (1998).** Tobacco mosaic virus infection induces severe morphological changes of the endoplasmic reticulum. *Proc Natl Acad Sci U S A* **95**, 11169–11174.
- Tomenius, K., Clapham, D. & Meshi, T. (1987).** Localization by immunogold cytochemistry of the virus-coded 30 K protein I plasmodesmata of leaves infected with tobacco mosaic virus. *Virology* **160**, 363–371.
- Tzfira, T., Rhee, Y., Chen, M. H., Kunik, T. & Citovsky, V. (2000).** Nucleic acid transport in plant–microbe interactions: the molecules that walk through the walls. *Annu Rev Microbiol* **54**, 187–219.
- Waigmann, E., Chen, M. H., Bachmaier, R., Ghoshroy, S. & Citovsky, V. (2000).** Regulation of plasmodesmal transport by phosphorylation of tobacco mosaic virus cell-to-cell movement protein. *EMBO J* **19**, 4875–4884.
- Watanabe, Y., Ogawa, T. & Okada, Y. (1992).** *In vivo* phosphorylation of the 30-Kda protein of tobacco mosaic virus. *FEBS Lett* **313**, 181–184.
- Wilson, C., Anglmayer, R., Vicente, O. & Heberle-Bors, E. (1995).** Molecular cloning, functional expression in *Escherichia coli*, and characterization of multiple mitogen-activated-protein kinases from tobacco. *Eur J Biochem* **233**, 249–257.
- Wolf, S., Deom, C. M., Beachy, R. & Lucas, W. J. (1991).** Plasmodesmatal function is probed using transgenic tobacco plants that express a virus movement protein. *Plant Cell* **3**, 593–604.
- Zhang, S. & Klessig, D. F. (1997).** Salicylic acid activates a 48-kD MAP kinase in tobacco. *Plant Cell* **9**, 809–824.