Biochemical and Biophysical Research Communications 426 (2012) 153–157

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

A conserved C-terminal motif is essential for self-interaction of *Barley stripe mosaic virus* China strain TGB3 protein

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ARTICLE INFO

Article history:
Received 3 August 2012
Available online 19 August 2012

Keywords:
Barley stripe mosaic virus China strain
TGB3
Self-interaction
Conserved C-terminal motif

ABSTRACT

The triple gene block (TGB) 3 protein is essential for the cell-to-cell movement of *Barley stripe mosaic virus* (BSMV). Previous studies have shown that TGB3, together with TGB2, facilitates the movement of TGB1 to the plasma membrane. However, the interactions among the three proteins (i.e., TGB3, TGB1, and TGB2) have not been thoroughly understood. The interactions of BSMV China strain (BSMV-CH) TGB3 with itself and with other two TGB proteins were investigated using a Gal4-based yeast two-hybrid system and pull-down assays. The results show that neither TGB1 nor TGB2 interacts with TGB3. However, self-interaction was detected for TGB3. The C-terminal 37 amino acids (amino acids 87–123) containing a conserved C-terminal motif were found required for the self-interaction of TGB3. The roles of the novel property of BSMV-CH TGB3 in virus cell-to-cell movement were discussed.

1. Introduction

Virus-coded movement proteins (MPs) are vital for the movement of plant viruses within and between cells. For some viruses, one MP is sufficient [1]. However, numerous other viruses possess two or three MPs for local or long distance transport [2,3]. Tripartite hordeiviruses contain three overlapping open reading frames known as triple-gene blocks (TGB) [4,5].

Barley stripe mosaic virus (BSMV), the typical member of the hordeiviruses, is a positive-sense, single-stranded (ss) RNA virus composed of three genome components designated as α, β, and γ. The α- and γ-RNAs are necessary for virus replication, whereas RNAβ is responsible for cell-to-cell movement [6,7]. One replicase protein, αa, which contains methyltransferase and helicase domains, is encoded by RNAα [8,9]. Another replicase protein, γa, is encoded by RNAγ and characterized by a polymerase (G-D-D) motif. A cysteine-rich protein encoded by RNAγ is expressed from a subgenomic (sg) RNA [5], designated as γb, which influences virulence, but is not required for replication or cell-to-cell movement [10–12]. The coat protein, βa, expressed directly from RNAβ, is dispensable for cell-to-cell movement and systemic movement [5,13,14]. On the other hand, the TGB proteins (TGBps) required for cell-to-cell movement are expressed via two sgRNAs of RNAβ. SgRNAb1 directs the synthesis of TGB1 protein, βb, characterized by a helicase motif, ATPase activity, and ssRNA, dsRNA, and nucleotides binding *in vitro* [15]. The TGB2 protein (βd), the TGB2′ minor translational read-through protein (βd′), and the TGB3 protein (βc) are expressed from sgRNAb2 [7,16]. Sequence analysis suggest that both TGB2 and TGB3 proteins are membrane-associated because they contain two hydrophobic membrane-spanning domains separated by a hydrophilic region [5].

The cell-to-cell movement of hordei-like viruses requires highly specific interactions among the cognate TGBps [17]. The mutations in the TGB2 and TGB3 of *Beet necrotic yellow vein virus* affect the level of TGB1 accumulation, indicating the existence of a highly coordinated control for the interactions among the elements of the movement machineries [12,18]. Previous results suggest that the cell-to-cell movement of *Potato mop-top virus* (PMTV) is regulated by TGB2 and TGB3 in relative amounts expressed in the cell [19–21]. Furthermore, the TGB2 and TGB3 of BSMV have been suggested to be required for the transport of TGB1 to and through the plasmodesmata [22]. However, the interactions between TGBps are still not thoroughly understood. In the present study, the interactions of BSMV China strain (CH) TGB3 with itself and with other two TGBps were investigated using a Gal4-based yeast two-hybrid system. The findings show that BSMV-CH TGB3 only interacts with itself and maps the self-interaction domain to 87–123 residues containing the conserved motif with a typical pentapeptide Y-Q-D-L-N.

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http://dx.doi.org/10.1016/j.bbrc.2012.08.056

2. Materials and methods

2.1. Plasmid constructs

TGB1, TGB2, and TGB3 were amplified using polymerase chain reaction (PCR) from a full-length cDNA of BSMV-CH RNAβ (GenBank Accession No. AY789694) with the primer pairs TGB1-F and TGB1-R for TGB1, TGB2-F and TGB2-R for TGB2, and TGB3-F and TGB3-R for TGB3, respectively (Table 1). The PCR products were ligated into the corresponding sites of pGADT7 and pGBKT7 vectors digested by the restriction enzymes according to the added primer pairs.

Deletion mutants of TGB3 were generated using PCR with the respective primer pairs TGB3ΔN40-F and TGB3-R for TGB3ΔN40, TGB3ΔN86-F and TGB3-R for TGB3ΔN86, TGB3ΔN123-F and TGB3-R for TGB3ΔN123, TGB3-F and TGB3ΔC32-R for TGB3ΔC32, and TGB3-F and TGB3ΔC69-R for TGB3ΔC69 (Table 1). To obtain the internal deletion mutant TGB3Δ87-123, primers TGB3-F and TGB3Δ87-123-R were used to amplify the upstream fragment of the deleted region (Table 1). Primers TGB3Δ87-123-F and TGB3-R were used to amplify the downstream fragment. Both PCR products were combined and amplified with primers TGB3-F and TGB3-R to render a read-through fragment bearing the deletion of residues 87–123 in TGB3. All PCR products of the TGB3 deletion mutants were digested with *Eco*RI/*Bam*HI, ligated with similarly digested pGADT7, and designated as pGADT7-TGB3ΔN40, pGADT7-TGB3ΔN86, pGADT7-TGB3ΔN123, pGADT7-TGB3ΔC32, pGADT7-TGB3ΔC69, and pGADT7-TGB3Δ87-123, respectively.

A T7 promoter was introduced at the 5′ terminal sequence of TGB3 using PCR from the full-length cDNA of BSMV RNAβ with primers TGBT7 (5′-GA**GAATTC**TAATACGACTCACTATA*ATGGCAATG CCTCATCCC*-3′; the *Eco*R I site is in boldface type, the T7 promoter is underlined, and the 5′ terminal sequence of TGB3 is in italics) and TGB3-R. The PCR products were ligated into the corresponding sites of the pMD18-T vector digested by the restriction enzymes according to the added primer pairs to construct pUC-TGB3. Similarly, His-Tag codons and a stop codon were introduced at the C-terminal TGB3 using PCR with the primers TGB3-F and TGB3-His (5′-GATATA**GGATCC**TCA*GTGGTGGTGGTGGTGGTGGTGCTTTTTTGA AGAAAGCAAGAG*-3′; the *Bam*H I site is in boldface type, the sequence complementary to the stop codon is underlined, and the sequence complementary to the His-Tag codons and to the 3′ terminal sequence of TGB3 is in italics). The PCR products were ligated into the corresponding sites of pET21b (Novagen) digested by the restriction enzymes according to the added primer pairs to construct pET-TGB3. All the constructs were confirmed by DNA sequencing.

Table 1
Primers used for the amplification of TGB proteins and the construction of the TGB3 mutants.

Primer	Sequence[a]
TGB1-F	5′GGAATTCCATATGGACATGACGAAGACTGTTG3′
TGB1-R	5′CGGGATCCTTATTGGCCTTGAACCAACTG3′
TGB2-F	5′GGAATTCATGAAGACCACAGTTGGTTCA3′
TGB2-R	5′TGGGATCCCTAGCCAATATCGCATAGTAATG3′
TGB3-F	5′GAGAATTCATGGCAATGCCTCATCCC3′
TGB3-R	5′CAGGATCCTCACTTTTTTGAAGAAAGCAAGAG3′
TGB3ΔN40-F	5′TAGAATTCGCGGAGAGGACTGTCAGG3′
TGB3ΔN86-F	5′GCGAATTCTACTTCTATCAAGATCTAAACT3′
TGB3ΔN123-F	5′GCGAATTCATGTGGGGAGGTTTAGTCAG3′
TGB3ΔC32-R	5′TAGGATCCCGGAGACTCCCCAAAAGG3′
TGB3ΔC69-R	5′GAGGATCCAGGACCAGTTTCCGTAGG3′
TGB3Δ87-123-F	5′ATGTGGGGAGGTTTAGTCAG3′
TGB3Δ87-123-R	5′CTGACTAAACCTCCCCACATAGGACCAGTTTCCGTAGG 3′

[a] Restriction enzyme sites used for cloning were underlined. All of the primers were synthesized by Invitrogen.

2.2. Yeast two-hybrid assay

The Gal4 transcription activator-based yeast two-hybrid system (MATCHMAKER GAL4 Two-Hybrid System 3, Clontech) was used to identify the interaction among the TGBps. The recombinant plasmids pGBKT7-TGB1, pGBKT7-TGB2, and pGBKT7-TGB3 were co-transformed with pGADT7 into *Saccharomyces cerevisiae* strain AH109 using the small-scale lithium acetate method (Clontech) to determine whether the expression of reporter genes could be activated. Subsequently, AH109 was co-transformed with pGADT7 and pGBKT7 recombinant DNAs. Cotransformants were plated on synthetic medium lacking leucine and tryptophan (-LW plate) to test for double transformation and lacking leucine, tryptophan, adenine, and histidine (-LWAH plate) for protein interaction. SV40 large T antigen$_{(84–708)}$ (pGADT7-T), which does not form complexes with many other proteins, interacts with murine p53$_{(72–390)}$ (pGBKT7-p53) or humanlamin C$_{(66–230)}$ (pGBKT7-Lamp) to serve as positive and negative controls, respectively.

2.3. β-Galactosidase assay

The β-galactosidase activity was evaluated using a colony-lift filter. Briefly, the colonies were re-streaked on a fresh -LWAH plate and then transferred to nitrocellulose filters, frozen in liquid nitrogen, thawed at room temperature, and finally assayed to measure the β-galactosidase activity by presoaking the filter in the substrate buffer (60 mM Na_2HPO_4, 40 mM NaH_2PO_4, 10 mM KCl, 1 mM $MgSO_4$, 20 mg/mL X-gal, pH 7.0) and by incubation at 30 °C. The times taken for the appearance of blue color and the intensity of the color after 6 h were both assessed.

2.4. In vitro translation

TGB3 and TGB3-His were translated *in vitro* from transcripts of pUC-TGB3 and pET-TGB3, respectively, in a TNT T7-coupled transcription/translation rabbit reticulocyte lysate (RRL) system (Promega, Madison, WI, USA), as described by the manufacturer. TGB3 was labeled with ^{35}S-methionine (Amersham Life Science, Buckinghamshire, UK), whereas the recombinant TGB3-His was unlabeled.

2.5. Pull-down assay

The *in vitro*-translated TGB3-His was immobilized on cobalt-sepharose beads (ProFound pull-down poly-His protein-protein interaction kit; Pierce Biotech, Boston, MA, USA) in wash buffer (WB) (25 mM Tris–HCl, pH 7.2, 0.15 M NaCl, and 40 mM imidazole) for 1 h at 4 °C. The beads were then washed in WB and incubated with ^{35}S-TGB3 at 4 °C for 2 h, also in WB. Assays were carried out using equimolar amounts of input material. After washing, the beads were boiled in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer. The protein bound to TGB3-His was resolved by 15% SDS-PAGE, and was detected by autoradiography.

3. Results

3.1. TGB3 self-interacts in vivo

The interactions of the BSMV-CH TGBps were examined using the yeast two-hybrid system based on the Gal4 transcription

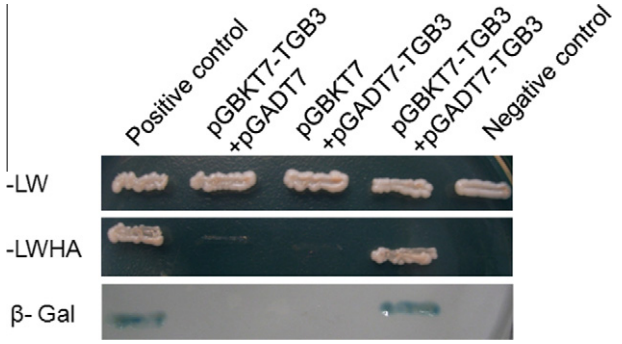

Fig. 1. Self-interaction of TGB3 *in vivo*. Plasmids (pGADT7-T and pGBKT7-53, positive control; pGADT7-T and pGBKT7-Lam, negative control; pGBKT7-TGB3 + pGADT7; pGBKT7 + pGADT7-TGB3; pGBKT7-TGB3 + pGADT7-TGB3) for yeast-two hybrid assay were transformed into yeast AH109, according to the manufacturer's instruction. Yeast cells transformed with bait and prey vector were streaked onto -LW plate (upper panel) and -LWAH plate (middle panel), respectively. lacZ activity was tested using β-galactosidase filter assay (β-Gal).

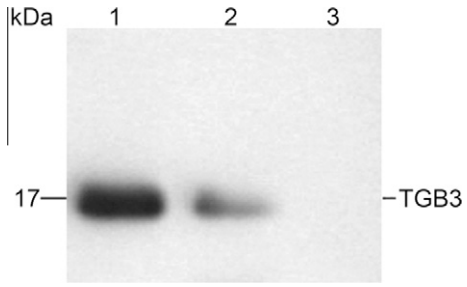

Fig. 2. Self-interaction of TGB3 *in vitro*. An autoradiograph showing the self-interaction of TGB3 as determined by pull-down assay. TGB3 (Lane 1) and TGB3-His were translated *in vitro* using the reticulocyte lysate system. TGB3 was translated in the presence of the recombinant His-tagged proteins and in the absence of ^{35}S-methionine. TGB3-His (Lane 2) was adsorbed on cobalt-sepharose beads and was incubated with ^{35}S-TGB3 (Lanes 2 and 3). After washing, the beads were boiled in an SDS-PAGE sample buffer, and the supernatant was resolved in 15% SDS-PAGE. The interacting proteins were detected by autoradiography.

activator. When the recombinant plasmids pGBKT7-TGB1, pGBKT7-TGB2, and pGBKT7-TGB3 were co-transformed with pGADT7 into the AH109 yeast cells, respectively, no clone was found growing on the -LWAH plate, indicating that the fusion proteins did not bind or transactivate nonspecifically to the lacZ reporter gene when expressed separately (data not shown). When the AH109 yeast cells were transformed with the combination of pGBKT7-TGB3 + pGADT7-TGB1, pGBKT7-TGB3 + pGADT7-TGB2 and pGBKT7-TGB3 + pGADT7-TGB3, respectively, the combination of pGBKT7-TGB3 and pGADT7-TGB3, like positive control, resulted in cell growth on the -LWAH plate. Similarly, when pGADT7-TGB3 was added into the AH109 cells with pGBKT7-TGB1, pGBKT7-TGB2 and pGBKT7-TGB3, respectively, no cell growth was observed on the -LWAH, except for co-transformants with pGADT7-TGB3 and pGBKT7-TGB3 (Fig. 1). The detection of the expression of lacZ gene through β-galactosidase activity showed that yeast transformants containing pGBKT7-TGB3 and pGADT7-TGB3, as well as the positive control, turned blue. These results clearly indicate that BSMV-CH TGB3 self-interacts specifically *in vivo*.

3.2. TGB3 self-interacts in vitro

To determine whether TGB3 has the intrinsic ability to self-interact *in vitro*, TGB3-His (recombinant TGB3 containing a C-terminal eight-histidine tag) and TGB3 translated *in vitro* in RRL were used for the pull-down assay. TGB3 was labeled with [^{35}S]-methionine, and the synthesis was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Fig. 2, lane 1). Recombinant TGB3-His, which was unlabeled during translation and pre-adsorbed to cobalt-sepharose beads, recruited the ^{35}S-labeled TGB3, as shown by the autoradiograph (Fig. 2, lane 2). Radiolabeled TGB3 did not bind to beads by itself (Fig. 2, lane 3). This result indicates the interaction between ^{35}S-TGB3 and TGB3-His, called the self-interaction of TGB3.

3.3. Mapping of the TGB3 self-interaction domain

To map the TGB3 domains involved in its self-interaction, three N-terminus deletion mutants of TGB3 (TGB3ΔN40, TGB3ΔN86, and TGB3ΔN123) were initially constructed from the N-terminus based on the previous report that the TGB3 of hordeiviruses contains two hydrophobic segments with a highly conserved interface between and the H-X-X-X-C-X-C-X-X-C consensus at the N-terminus [5]. These mutants were tested to determine their interactions with the full-length TGB3 using the Gal4-based yeast two-hybrid system. As shown in Fig. 3, TGB3ΔN40 and TGB3ΔN86, as well as the full-length TGB3, interact with the full-length TGB3. This finding indicated that the region between amino acids 1–86 does not contribute to BSMV-CH TGB3 self-interaction. The co-transformants with pGADT7-TGB3ΔN123 and pGBKT7-TGB3 resulted in the formation of yeast colonies on -LWAH plate, indicating that the region of interest in BSMV-CH TGB3 self-interaction is located in amino acids 87–123. To further confirm this result, three C-terminus deletion mutants of TGB3 (TGB3ΔC32, TGB3ΔC69, and TGB3Δ87-123) were constructed. Only TGB3ΔC32, which contains amino acids 87–123, interacts with the full-length TGB3, whereas TGB3ΔC69 and TGB3Δ87-123 do not. Therefore, the C-terminal domain of residues 87–123, which contains the highly conserved motif, is required for binding. Other regions are not involved in the interaction.

4. Discussion

All three TGBps are essential for the cell-to-cell movement of *White clover mosaic virus* [23], *Potato virus X* [20], *Bamboo mosaic virus* (BaMV) [17], *Peanut clump virus* [24], and BSMV [22]. To activate the cell-to-cell movement function of these viruses, the TGBps need to interact with each other, either directly or indirectly [1]. In the case of BSMV, the site-specific mutations introduced into the six conserved regions of the TGB1 helicase domain affect the expression of the TGB2 and/or TGB3 proteins. Cell-to-cell movement requires a TGB1 association facilitated by the TGB2 and TGB3 proteins with cytoplasmic membranes [22]. Although the interaction between the TGB2 and TGB3 of BSMV ND18 was detected using a yeast two-hybrid system, the self-interaction of TGB3 was not detected [25]. In the current study, direct interactions of BSMV-CH TGB3 with TGB1 and TGB2, as well as with itself, were carried out using a Gal4-based yeast two-hybrid system. Our results suggest that BSMV-CH TGB3 only interacts with itself, and not with TGB1 or TGB2. The inconsistencies in the results may be attributed to the 5 amino acids difference of TGB3 sequence between BSMV-CH and BSMV ND18 or to the use of a different yeast strain in the yeast two-hybrid system. The major domain required for self-interaction is located in the C-terminal region of TGB3 protein and, more precisely, within the 87–123 amino acids, which contain the highly conserved domain of TGB3. The specific heterologous TGB3-TGB1 interaction of BSMV ND18 was detected using affinity chromatography [25]. No test was conducted to evaluate this affinity, so interaction may exist between TGB3 and TGB1 of BSMV-CH.

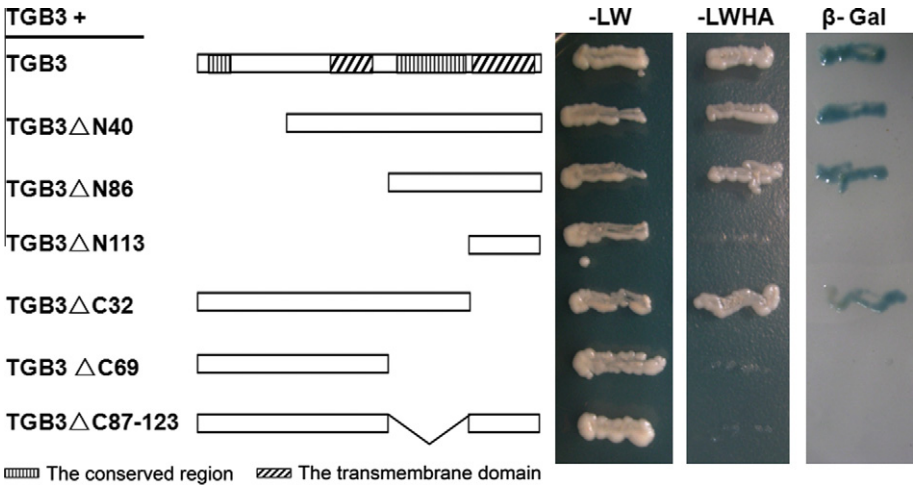

Fig. 3. Mapping of TGB3 domains required for its self-interaction with the yeast two-hybrid system. *S. cerevisiae* strain AH109 was co-transformed with the yeast two-hybrid constructs encoding TGB3 fused to GAL4BD, and TGB3 or TGB3 deletion mutants fused to GAL4AD. The transformants obtained were streaked onto -LW plate and -LWAH plate, respectively. lacZ activity was tested using β-galactosidase filter assay (β-Gal).

Previous studies have shown that the self-interaction of viral proteins is crucial for many of their functions in the viral life cycle [26–29], and self-interaction is likely critical for their ability to interact with multiple cellular proteins simultaneously [29,30]. The primary function of TGB3 involves intracellular movement to facilitate the passage of the viral RNP complex to and through the plasmodesmata [2,22,31–33]. *Poa semilatent hordeivirus* (PSLV) TGBp2 associates mostly with the endoplasmic reticulum (ER), whereas TGBp3 has been found in membrane bodies of different sizes located at the cell periphery along the plasma membrane [5]. The co-expression of TGBp2 and TGBp3 of PSLV or BaMV demonstrated that TGBp3 directs the transport of TGBp2 from the ER structures to the peripheral bodies [34]. Examination of the PSLV GFP-18K-expressing epidermal cells of transgenic plants under high magnification revealed that the peripheral fluorescent bodies located at the cell wall represent twin structures consisting of pairs of disconnected bodies located on the opposite sides of the cell wall [35]. Similar twin bodies were found in plant tissues infected with a tobacco mosaic virus vector expressing a GFP-fused TGBp3 protein of PMTV [36]. Although self-interaction has been detected between all three PMTV TGBps, only TGB1 self-interaction could be detected *in vitro* [37]. In the present study, the self-interaction of BSMV-CH TGB3 was confirmed *in vivo* and *in vitro*. Therefore, this characteristic may be related to the formation of opposing pairs of fluorescent spots near the cell walls in GFP-TGBp3-infected cells, which correlated with the previous report showing that the polymerization of BaMV TGBp3 was involved in forming punctate structures during peripheral body biogenesis [34]and reducing the polymerization of BaMV TGBp3 affected its ER tubule localization [38].

Mapping of the self-interaction domain showed the binding region located in the C-terminal of residues 87–123, which contain the motif Y-Q-D-L-N-X13-P-X-V-I-X6-P-X-G (where X is any amino acid residue) conserved in hordei-, pomo-, and pecluviruses. Y-Q-D-L-N, which contains a putative Tyr-based sorting signal based on the motif Y-X-X-F (F represents an amino acid with bulky hydrophobic side chain) [39], usually occurs in the cytoplasmic tails of transmembrane proteins and plays an important role in the internalization and targeting of proteins to subcellular compartments, such as endosomes, lysosomes, or other organelles [39,40]. Previous studies have found Tyr-based motifs in several animal viruses that enter the cells by endocytosis [41], as well as in the MP of grapevine fan leaf virus and in the MPs of several nepoviruses [42]. The disruption of this motif in PSLV TGB3 by the insertion of four amino acids (R-S-T-D) between the D and L residues not only abolished the inability of PSLV TGB3 to be transported to the cell surface-associated compartments to form the cell peripheral bodies and to target PSLV TGB2 in the cell periphery, but also blocked the protein incorporation into high-molecular-mass complexes [38]. Both deletion mutants and site-directed substitutions in the P-X-V-I-X6-P-X-G region of PSLV TGB3 showed the same results [5,43,44]. Similarly, the mutation Y-Q-D-L-N to G-Q-D-G-N caused a change in the subcellular localization of PTMV TGB3, eliminating the ER localization and the presence of small motile granules [37]. In our experiments, the crucial role of the motif Y-Q-D-L-N-X13-P-X-V-I-X6-P-X-G has been found in the BSMV-CH TGB3 self-interaction. No bioassay experiments were conducted in the present research to demonstrate the biological function of this self-interaction, but previous report showed that mutations in the TGB3 of BSMV type strain affect the cell-to-cell movement of the virus and the localization of TGB2 [25,45]. Therefore, the current study led to the discovery of a new property of BSMV TGB3 that has an important role in virus cell-to-cell movement.

Acknowledgments

The authors would like to thank Dr. Helen Hull-Sanders for her critical reading of the manuscript. This work was supported by the National Natural Science Foundation of China (30370060).

References

[1] J. Verchot-Lubicz, L. Torrance, A.G. Solovyev, S.Y. Morozov, A.O. Jackson, D. Gilmer, Varied movement strategies employed by triple gene block-encoding viruses, Mol. Plant Microbe Interact. 23 (2010) 1231–1247.

[2] T.J. Lough, N.E. Netzler, S.J. Emerson, P. Sutherland, F. Carr, D.L. Beck, W.J. Lucas, R.L. Forster, Cell-to-cell movement of potexviruses: evidence for a ribonucleoprotein complex involving the coat protein and first triple gene block protein, Mol. Plant Microbe Interact. 13 (2000) 962–974.

[3] S. Morozov, O.N. Fedorkin, G. Juttner, J. Schiemann, D.C. Baulcombe, J.G. Atabekov, Complementation of a potato virus X mutant mediated by bombardment of plant tissues with cloned viral movement protein genes, J. Gen. Virol. 78 (Pt 8) (1997) 2077–2083.

[4] S. Morozov, V.V. Dolja, J.G. Atabekov, Probable reassortment of genomic elements among elongated RNA-containing plant viruses, J. Mol. Evol. 29 (1989) 52–62.

[5] A.G. Solovyev, E.I. Savenkov, A.A. Agranovsky, S.Y. Morozov, Comparisons of the genomic cis-elements and coding regions in RNA beta components of the hordeiviruses barley stripe mosaic virus, lychnis ringspot virus, and poa semilatent virus, Virology 219 (1996) 9–18.

[6] I.T. Petty, R. French, R.W. Jones, A.O. Jackson, Identification of barley stripe mosaic virus genes involved in viral RNA replication and systemic movement, EMBO J. 9 (1990) 3453–3457.
[7] H. Zhou, A.O. Jackson, Expression of the barley stripe mosaic virus RNA beta "triple gene block", Virology 216 (1996) 367–379.
[8] G. Gustafson, S.L. Armour, G.C. Gamboa, S.G. Burgett, J.W. Shepherd, Nucleotide sequence of barley stripe mosaic virus RNA alpha: RNA alpha encodes a single polypeptide with homology to corresponding proteins from other viruses, Virology 170 (1989) 370–377.
[9] J.J. Weiland, M.C. Edwards, Evidence that the alpha a gene of barley stripe mosaic virus encodes determinants of pathogenicity to oat (*Avena sativa*), Virology 201 (1994) 116–126.
[10] R.G. Donald, A.O. Jackson, The barley stripe mosaic virus gamma b gene encodes a multifunctional cysteine-rich protein that affects pathogenesis, Plant Cell 6 (1994) 1593–1606.
[11] R.G. Donald, A.O. Jackson, RNA-binding activities of barley stripe mosaic virus gamma b fusion proteins, J. Gen. Virol. 77 (Pt 5) (1996) 879–888.
[12] J.N. Bragg, D.M. Lawrence, A.O. Jackson, The N-terminal 85 amino acids of the barley stripe mosaic virus gammab pathogenesis protein contain three zinc-binding motifs, J. Virol. 78 (2004) 7379–7391.
[13] G. Gustafson, S.L. Armour, The complete nucleotide sequence of RNA beta from the type strain of barley stripe mosaic virus, Nucleic Acids Res. 14 (1986) 3895–3909.
[14] I.T. Petty, A.O. Jackson, Mutational analysis of barley stripe mosaic virus RNA beta, Virology 179 (1990) 712–718.
[15] R.G. Donald, D.M. Lawrence, A.O. Jackson, The barley stripe mosaic virus 58-kilodalton beta(b) protein is a multifunctional RNA binding protein, J. Virol. 71 (1997) 1538–1546.
[16] J.A. Johnson, J.N. Bragg, D.M. Lawrence, A.O. Jackson, Sequence elements controlling expression of barley stripe mosaic virus subgenomic RNAs in vivo, Virology 313 (2003) 66–80.
[17] M.K. Lin, C.C. Hu, N.S. Lin, B.Y. Chang, Y.H. Hsu, Movement of potexviruses requires species-specific interactions among the cognate triple gene block proteins, as revealed by a trans-complementation assay based on the bamboo mosaic virus satellite RNA-mediated expression system, J. Gen. Virol. 87 (2006) 1357–1367.
[18] E. Lauber, C. Bleykasten-Grosshans, M. Erhardt, S. Bouzoubaa, G. Jonard, K.E. Richards, H. Guilley, Cell-to-cell movement of beet necrotic yellow vein virus: I. Heterologous complementation experiments provide evidence for specific interactions among the triple gene block proteins, Mol. Plant Microbe Interact. 11 (1998) 618–625.
[19] C. Bleykasten-Grosshans, H. Guilley, S. Bouzoubaa, K.E. Richards, G. Jonard, Independent expression of the first two triple gene block proteins of beet necrotic yellow vein virus complements virus defective in the corresponding gene but expression of the third protein inhibits viral cell-to-cell movement, Mol. Plant Microbe Interact. 10 (1997) 240–246.
[20] Y. Yang, B. Ding, D.C. Baulcombe, J. Verchot, Cell-to-cell movement of the 25 K protein of potato virus X is regulated by three other viral proteins, Mol. Plant Microbe Interact. 13 (2000) 599–605.
[21] A. Tamai, T. Meshi, Cell-to-cell movement of Potato virus X: the role of p12 and p8 encoded by the second and third open reading frames of the triple gene block, Mol. Plant Microbe Interact. 14 (2001) 1158–1167.
[22] D.M. Lawrence, A.O. Jackson, Interactions of the TGB1 protein during cell-to-cell movement of barley stripe mosaic virus, J. Virol. 75 (2001) 8712–8723.
[23] D.L. Beck, P.J. Guilford, D.M. Voot, M.T. Andersen, R.L. Forster, Triple gene block proteins of white clover mosaic potexvirus are required for transport, Virology 183 (1991) 695–702.
[24] E. Herzog, O. Hemmer, S. Hauser, G. Meyer, S. Bouzoubaa, C. Fritsch, Identification of genes involved in replication and movement of peanut clump virus, Virology 248 (1998) 312–322.
[25] H.S. Lim, J.N. Bragg, U. Ganesan, D.M. Lawrence, J. Yu, M. Isogai, J. Hammond, A.O. Jackson, Triple gene block protein interactions involved in movement of barley stripe mosaic virus, J. Virol. 82 (2008) 4991–5006.
[26] M. Haas, A. Geldreich, M. Bureau, L. Dupuis, V. Leh, G. Vetter, K. Kobayashi, T. Hohn, L. Ryabova, P. Yot, M. Keller, The open reading frame VI product of Cauliflower mosaic virus is a nucleocytoplasmic protein: its N terminus mediates its nuclear export and formation of electron-dense viroplasms, Plant Cell 17 (2005) 927–943.
[27] Y.L. Khu, E. Koh, S.P. Lim, Y.H. Tan, S. Brenner, S.G. Lim, W.J. Hong, P.Y. Goh, Mutations that affect dimer formation and helicase activity of the hepatitis C virus helicase, J. Virol. 75 (2001) 205–214.
[28] Y. Takemoto, T. Hibi, Self-interaction of ORF II protein through the leucine zipper is essential for Soybean chlorotic mottle virus infectivity, Virology 332 (2005) 199–205.
[29] M. Tanaka, A. Yokoyama, M. Igarashi, G. Matsuda, K. Kato, M. Kanamori, K. Hirai, Y. Kawaguchi, Y. Yamanashi, Conserved region CR2 of Epstein-Barr virus nuclear antigen leader protein is a multifunctional domain that mediates self-association as well as nuclear localization and nuclear matrix association, J. Virol. 76 (2002) 1025–1032.
[30] S. Harada, R. Yalamanchili, E. Kieff, Epstein-Barr virus nuclear protein 2 has at least two N-terminal domains that mediate self-association, J. Virol. 75 (2001) 2482–2487.
[31] A.A.J. Zamyatnin, A.G. Solovyev, E.I. Savenkov, A. Germundsson, M. Sandgren, J.P.T. Valkonen, S.Y. Morozov, Transient coexpression of individual genes encoded by the triple gene block of Potato mop-top virus reveals requirements for GBp1 trafficking, Mol. Plant Microbe Interact. 17 (2004) 921–930.
[32] M. Erhardt, M. Morant, C. Ritzenthaler, C. Stussi-Garaud, H. Guilley, K. Richards, G. Jonard, S. Bouzoubaa, D. Gilmer, P42 movement protein of Beet necrotic yellow vein virus is targeted by the movement proteins P13 and P15 to punctate bodies associated with plasmodesmata, Mol. Plant Microbe Interact. 130 (2000) 520–528.
[33] S. Santa Cruz, A.G. Roberts, D.A.M. Prior, S. Chapman, K.J. Oparka, Cell-to-cell and phloem-mediated transport of potato virus X: the role of virions, Plant Cell 10 (1998) 495–510.
[34] S.C. Lee, C.H. Wu, C.W. Wang, Traffic of a viral movement protein complex to the highly curved tubules of the cortical endoplasmic reticulum, Traffic 11 (2010) 912–930.
[35] E.N. Gorshkova, T.N. Erokhina, T.A. Stroganova, N.E. Yelina, A.A. Zamyatnin Jr., N.O. Kalinina, J. Schiemann, A.G. Solovyev, S.Y. Morozov, Immunodetection and fluorescent microscopy of transgenically expressed hordeivirus TGBp3 movement protein reveals its association with endoplasmic reticulum elements in close proximity to plasmodesmata, J. Gen. Virol. 84 (2003) 985–994.
[36] G.H. Cowan, F. Lioliopoulou, A. Ziegler, L. Torrance, Subcellular localisation, protein interactions, and RNA binding of Potato mop-top virus triple gene block proteins, Virology 298 (2002) 106–115.
[37] S. Haupt, G.H. Cowan, A. Ziegler, A.G. Roberts, K.J. Oparka, L. Torrance, Two plant-viral movement proteins traffic in the endocytic recycling pathway, Plant Cell 17 (2005) 164–181.
[38] E.A. Shemyakina, T.N. Erokhina, E.N. Gorshkova, J. Schiemann, A.G. Solovyev, S.Y. Morozov, Formation of protein complexes containing plant virus movement protein TGBp3 is necessary for its intracellular trafficking, Biochimie 93 (2011) 742–748.
[39] M.S. Marks, H. Ohno, T. Kirchnausen, J.S. Bonracino, Protein sorting by tyrosine-based signals: adapting to the Ys and wherefores, Trends Cell Biol. 7 (1997) 124–128.
[40] J.S. Bonifacino, E.C. Dell'Angelica, Molecular bases for the recognition of tyrosine-based sorting signals, J. Cell Biol. 145 (1999) 923–926.
[41] S.B. Sieczkarski, G.R. Whittaker, Dissecting virus entry via endocytosis, J. Gen. Virol. 83 (2002) 1535–1545.
[42] C. Laporte, G. Vetter, A.M. Loudes, D.G. Robinson, S. Hillmer, C. Stussi-Garaud, C. Ritzenthaler, Involvement of the secretory pathway and the cytoskeleton in intracellular targeting and tubule assembly of Grapevine fanleaf virus movement protein in tobacco BY-2 cells, Plant Cell 15 (2003) 2058–2075.
[43] A.G. Solovyev, T.A. Stroganova, A.A. Zamyatnin Jr., O.N. Fedorkin, J. Schiemann, S.Y. Morozov, Subcellular sorting of small membrane-associated triple gene block proteins: TGBp3-assisted targeting of TGBp2, Virology 269 (2000) 113–127.
[44] M.V. Schepetilnikov, A.G. Solovyev, E.N. Gorshkova, J. Schiemann, A.I. Prokhnevsky, V.V. Dolja, S.Y. Morozov, Intracellular targeting of a hordeiviral membrane-spanning movement protein: sequence requirements and involvement of an unconventional mechanism, J. Virol. 82 (2008) 1284–1293.
[45] H.S. Lim, J.N. Bragg, U. Ganesan, S. Ruzin, D. Schichnes, M.Y. Lee, A.M. Vaira, K.H. Ryu, J. Hammond, A.O. Jackson, Subcellular localization of the barley stripe mosaic virus triple gene block proteins, J. Virol. 83 (2009) 9432–9448.