

TGB2 movement proteins of the Hordei type localise to chloroplasts, the sites of virus replication, indicating a novel functional role

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Summary

Barley stripe mosaic (BSMV) and potato-top virus (PMTV) genomes contain modules of three overlapping genes called the triple gene block (TGB) of the hordeivirus type. The TGB proteins are essential for movement. In addition, BSMV encodes a multi-functional, cysteine rich protein called γ B that has RNA silencing suppression and pathogenicity enhancing activities.

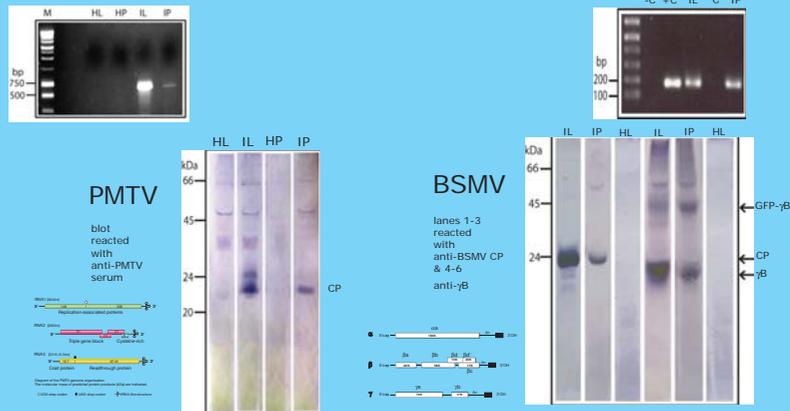
Studies of the expression and localisation of fluorescent-protein tagged TGB2 proteins and γ B using confocal microscopy have revealed that in addition to localisations in the ER, motile granules and at the cell periphery; later in expression, the TGB2 proteins associated with components of the endocytic pathway. Surprisingly, they also associated with plastids. Observations of thin sections of infected leaves suggested that plastids were sites of virus replication and the electron microscopy was confirmed by RT-PCR and Western blots of virus infected leaves and plastid preparations. The results suggest that the TGB2 and γ B play a role in supporting virus replication in the later stages of infection after the exit of viral RNP complexes from the cell; possibly by assisting recruitment and/or assembly and establishment of replication complexes for the production of virions.

Ref: Torrance et al (2006) J Gen Virol. 87, 2403 & unpubl. results

Results

Detection of virus RNA and CP in plastids

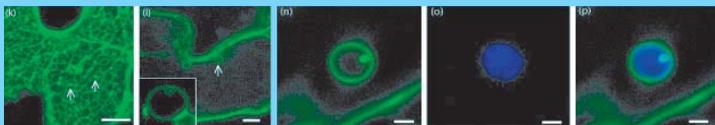
Plastid preparations (P) from BSMV or PMTV infected (I) leaves were tested by RT-PCR and western blots. RT-PCR showed presence of PMTV & BSMV RNA and westerns revealed presence of viral CPs and γ B.



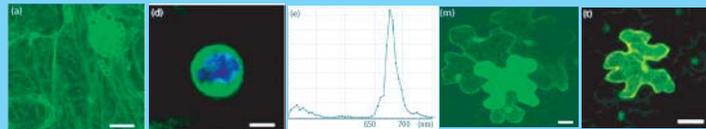
Confocal microscopy

BSMV:

35S-GFP-TGB2 expressed in epidermal cells of *N. benthamiana* localises to ER, motile granules (k, arrowed) and the membranes of pleiomorphic vesicles (l) but when introduced into cells together with viral RNA GFP-TGB2 also localises to the chloroplast envelope (n,o,p; chlorophyll autofluorescence coloured blue).

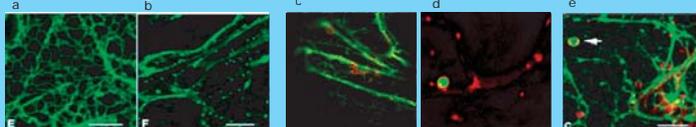


35S-GFP-gB is cytosolic but localises to chloroplasts in the presence of RNA α and γ (a, d). Chloroplast localisation is independent of the presence RNA β (TGB encoding RNA). (e) lambda scan showing peak at 680 nm of chlorophyll a autofluorescence (m) 35S-GFP-TGB2 moves to adjacent cells (t) No association of GFP-TGB2 and mRFP- γ B

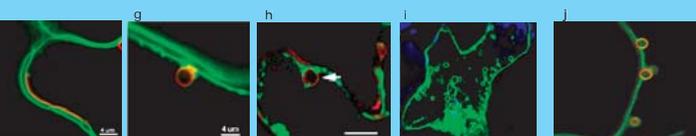


PMTV:

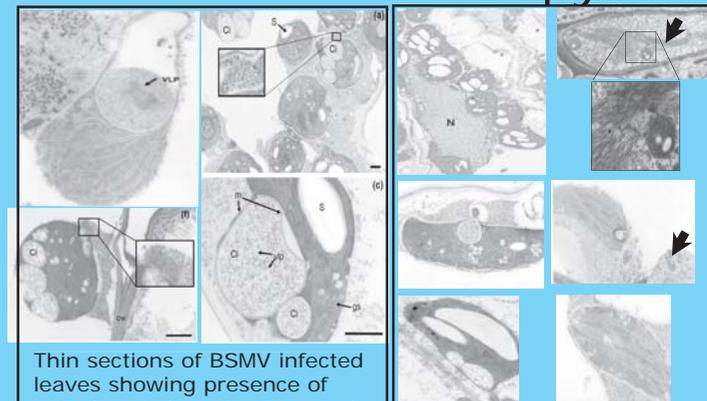
Transiently expressed GFP or mRFP-TGB2 localises to the ER and motile granules (a,b) it also localises to the membranes of at least two populations of vesicle like compartments, motile small c. 2 μ m (c) and less motile c. 4 μ m round structures (d). Analysis by lambda scanning showed that some (but not all) of the c. 4 μ m compartments were chloroplasts (chlorophyll autofluorescence artificially coloured green).



35S-mRFP-TGB2 was shown to associate with components of the endocytic pathway by co-expression and co-localisation studies with endocytic markers. (e) co-localisation with the endosomal stain FM 4-64; (f, g) 35S-mRFP-TGB2 in epidermal cells of transgenic plants expressing GFP targeted to the plasma membrane; TGB2 localises to patches of PM over pit field (f; arrow) and membrane of vesicle (g); 35S-mRFP-TGB2 colocalises in the membrane of vesicles with GFP-Ara7 (At RabF2b) a Rab 5 homologue and endosomal marker(h); expression of GFP-Ara7 in epidermal cells, note labelling on vesicle membranes but none associated with chloroplasts (i; coloured blue). 35S-mRFP-TGB2 also co-localises in membranes of PM and vesicles with GFP-RME8 from *C. elegans* (j). The subcellular localisation of 35S-mRFP-TGB2 was identical when expressed in the presence of a PMTV GFP-TGB1 tagged reporter clonemutated to insert a stop codon immediately in front of the ORF for TGB2 (not shown). Note 35S-mRFP-TGB2 of PMTV does not require viral RNA for localisation to plastids.



Electron microscopy



Thin sections of BSMV infected leaves showing presence of cytoplasmic invaginations and inclusions (CI) in distorted chloroplasts and presence of virus-like particles (VLPs) in CI and appressed to outer chloroplast envelope

Thin sections of PMTV infected leaves showing distorted chloroplasts with cytoplasmic invaginations. Virus-like particles in cytoplasm (arrowed)



35S-mRFP-TGB2 co-localises in the membrane of vesicles with the Rab 5 homologue GFP-Ara7 (At RabF2b) and recruits Ara7 to the chloroplast envelope

CLSM methods: BSMV cDNA clones were based on strain ND18 (Petty et al, 1988); clone RNA γ was modified so that green fluorescent protein was fused to γ B (GFP-2A- γ B or γ B-GFP). The clones were used in different combinations together with RNA α , RNA γ , 35S-GFP- γ B and 35S-GFP-TGB2 (cloned in pRTL2 under the control of the 35S promoter). A PMTV GFP-reporter clone (Savenkov et al., 2003, J Gen Virol 84, 1001) was modified to delete the ORF for TGB2 and was used in experiments singly or together with 35S-mRFP-TGB2. The constructs were introduced into epidermal cells of barley and *N. benthamiana* and examined by CLSM 1-2 days post bombardment.

Acknowledgements

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