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 mop-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 β8 that has RNA silencing suppression and pathogenicity enhancing activities.

Studies of the expression and localisation of fluorescent-protein tagged TGB2 proteins and β8 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 confirmatory by RT-PCR and Western blots of virus infected leaves and plastid preparations. The results suggest that the TGB2 and β8 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.


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 β8.

Confocal microscopy

BSMV:

35S-GFP-TGB2 expressed in epidermal cells of N. benthamiana localises to ER, motile granules (a, arrowed) and the membranes of pleomorphic vesicles (b) but when introduced into cells together with viral RNA GFP-TGB2 also localises to the chloroplast envelope (c, blue).


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 associated to outer chloroplast envelope

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

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