The role of Potato mop-top virus TGB1 movement protein in cell-cell and long-distance transport.

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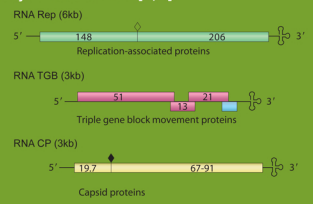
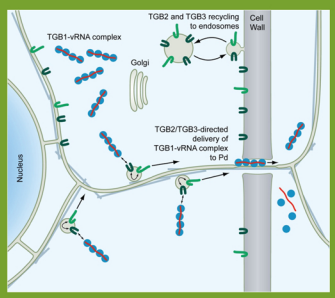
Introduction

Potato Mop-Top Virus.

PMTV encodes a triple gene block (TGB) movement module. TGB act in a coordinated manner to facilitate movement of the virus genome. Some features of the movement mechanism are conserved among TGB encoding viruses but there are major differences [1,2].

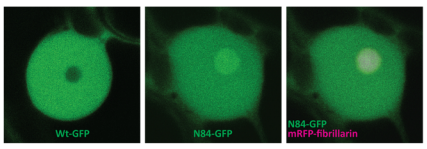


PMTV genomic RNA can move systemically in the absence of coat protein presumably as a viral ribonucleoprotein complex (vRNP)[2-4]. Experimental evidence supports a model (below) where membrane associated TGB2 and TGB3 interact with and facilitate transport of vRNP (comprising TGB1 with viral RNA) on the actin-ER network. The whole complex targets and gates plasmodesmata (PD) allowing passage of vRNP to the neighbouring cell while TGB2 and TGB3 are recycled via the endocytic pathway [4,5]. This poster focuses on the role and interactions of TGB1 with TGB2/TGB3 in cell-to-cell spread. The data suggest an additional role for passage of TGB1 through the nucleus and nucleolus to facilitate systemic movement.



Model for local cell-cell movement. A viral ribonucleoprotein complex consisting of viral RNA bound by TGB1 is shuttled to PD by the TGB2/3 complex and then moves into the adjoining cell.

The N-terminal 84 amino acids of TGB1 contain a nucleolar targeting signal



Acknowledgements
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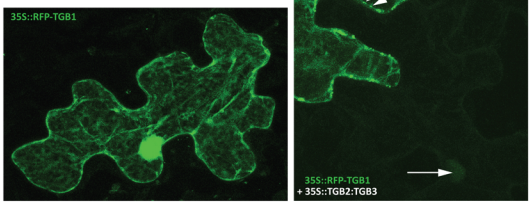
References
[1] Morozov & Solovyev (2003) J Gen Virol 84, 1351-1366; [2] Torrance et al. (2009) MPMI 22 381-390; McGeachy & Barker (2000) MPMI 13, 125-128; [4]Lim et al. (2008) J Virol 82, 4991-5006; [5]Verchot-Lubicz et al. (2010) MPMI 10, 1231-1247; [6]Wright et al. (2010) MPMI 23, 1486-1497 [7]Oparka & Turgeon (1999) Plant Cell 11, 739-750

Results :

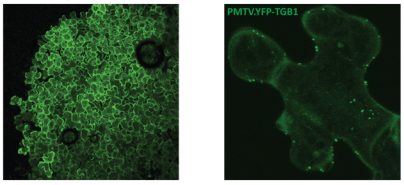
35S-expressed TGB1

By itself shows a nuclear and cytoplasmic distribution.

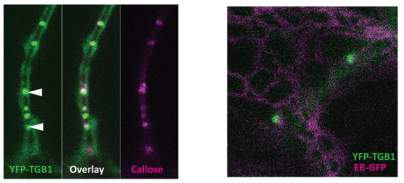


However, co-expression of unlabeled TGB2 and TGB3 from a bicistronic plasmid (35S::TGB2:TGB3), resulting in a ~10:1 TGB2/TGB3 ratio similar to viral infection , resulted in targeting of TGB1 to plasmodesmata (arrowheads). The protein also trafficked intercellularly in the presence of TGB2 and 3 (arrows).

Virus-expressed TGB1

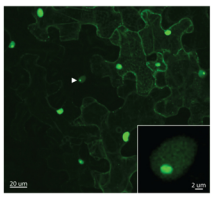


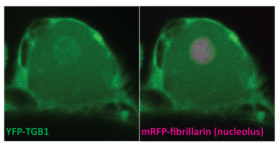
A PMTV clone with fluorescent protein fused to the N-terminus of TGB1 induces local lesions but is incompetent for long-distance movement (LDM). In cells at the leading edge TGB1 is seen in PD and moving spots on the ER.

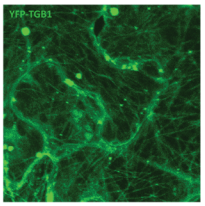


Redistribution of TGB1 behind the infection front

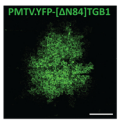
Within two to three cells of the leading edge YFP-TGB1 accumulates in the nucleolus and on microtubules (MT) . Nucleolar enrichment and occasional labelling of MT were also observed with 35S-expressed YFP-TGB1.



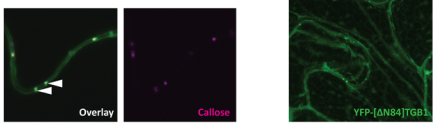




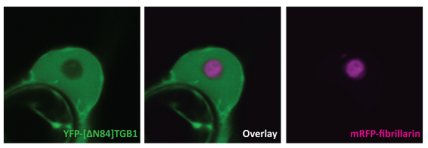
Role of TGB1 N-terminus in protein localisation



We imaged the subcellular localisation of the virus- and 35S-expressed mutant [ΔN84]TGB1 protein.

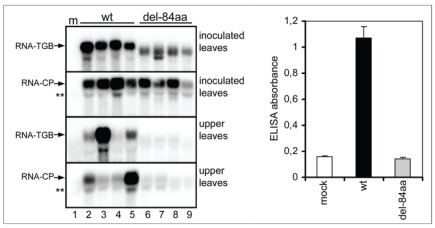


The deletion did not affect local cell-to-cell movement or PD localisation of TGB1. However, no association with the nucleolus or MT was observed for [ΔN84]TGB1.



Role of the TGB1 N-terminus in viral long-distance movement

Because fusion of a fluorescent protein to the N-terminus of TGB1 renders the virus incapable of long-distance movement through the phloem, we investigated the role of the N-terminus on LDM deleting the N-terminal 84 amino acids.
The ΔN84-deletion abolished long distance movement, both in the presence and absence of RNA CP.

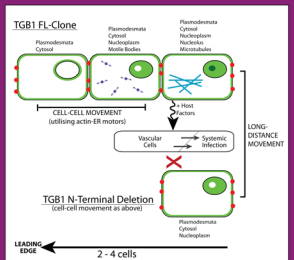


Northern blot analysis of progeny viral RNAs accumulated in *Nicotiana benthamiana* plants inoculated with the mutant of RNA-TGB transcripts along with wild-type (wt) RNA 1 and RNA-CP transcripts. Detection of PMTV CP by enzyme-linked immunosorbent assay.

Disussion

Interaction with nuclear factors?

TGB1 interacts with the read-through product of the PMTV coat protein gene (CP-RT) and both proteins are thought to reside at the 5′ end of encapsidated PMTV particles [2]. [ΔN84]TGB1 still interacts with CP-RT in yeast-two-hybrid experiments ([6] not shown).
The role of the TGB1 N-terminus in LDM may be related to interactions with host proteins.
The accumulation of Fluorescent protein-fused TGB1 in the nucleolus could indicate that the native protein shuttles through this compartment to recruit host proteins required for LDM.



Systemic movement requires entry into the vasculature, which comprises a major barrier [7]. Many plant viruses employ distinct mechanisms to achieve local and systemic intercellular movement. Because of the potential delay in vascular entry, events observed behind the leading edge of an epidermal infection site may correspond to the switch to systemic movement.