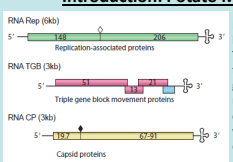
The coordinated action of potato mop-top virus triple gene block movement proteins in viral cell-to-cell transport

Kathryn M. Wright[1], Graham Cowan[1], Nina Lukhovitskaya[2], Jens Tilsner[1], Alison G. Roberts[1], Eugene Savenkov[2] and Lesley Torrance[1]

[1]Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, U.K.; [2]Department of Plant Biology and Forest Genetics, Uppsala BioCenter SLU, Box 7080, SE-750 07 Uppsala, Sweden

Introduction: Potato Mop-Top Virus

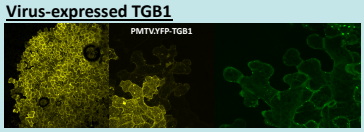


PMTV encodes a triple gene block (TGB) movement module. TGB act in a coordinated manner to facilitate movement of virus genome. Some features of the movement mechanism are conserved among TGB encoding viruses but there are major differences [1,2].

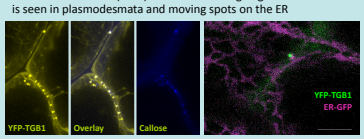
PMTV genomic RNA can move systemically in the absence of coat protein presumably as a viral ribonucleoprotein complex (vRNP)[2-4].

Experimental evidence supports a model (see below) where membrane associated TGB2 and TGB3 interact with and facilitate transport, on the actin-ER network, of vRNP (comprising TGB1 with viral RNA). 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.

Virus-expressed TGB1



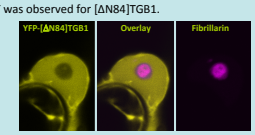
A PMTV clone with a fluorescent protein fused to the N-terminus of TGB1 induces local lesions but is incompetent for systemic long-distance movement (LDM). In cells at leading edge TGB1 is seen in plasmodesmata and moving spots on the ER



Role of TGB1 N-terminus in protein localisation

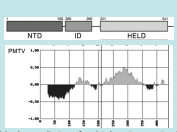
We also imaged the subcellular localisation of the virus- and 35S-expressed mutant [ΔN84]TGB1 protein.

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.



Possible function of the TGB1 N-terminus

TGB1 has three distinct RNA binding domains: The disordered N-terminal domain (NTD), the intermediate domain (ID), and the RNA helicase domain (HELD) [7]

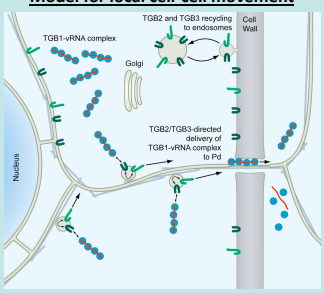


NTD and ID non-sequence specific ssRNA & dsRNA binding

HELD = NTPase/helicase domain (conserved in potex and hordei-like TGB1s)

It is unlikely that fusion of a fluorescent protein at the N-terminus or deletion of the first 84 amino acids abolished TGB1 RNA-binding because:
- The virus still moved cell-to-cell
- [ΔN84]TGB1 still retains two RNA binding domains
- LDM was affected also in the presence of coat protein, where PMTV is believed to move systemically in an encapsidated form [2]

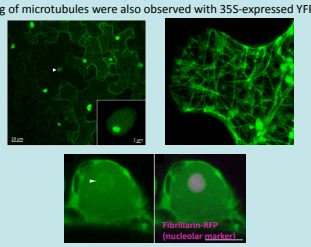
Model for local cell-cell movement



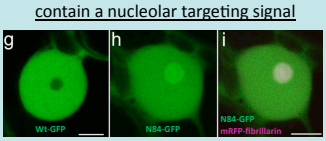
A viral ribonucleoprotein complex (vRNP) consisting of viral RNA bound by TGB1 [4,5] is shuttled to plasmodesmata by the TGB2/3 complex and then moves into the adjoining cell

Redistribution of TGB1 behind the infection front

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



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

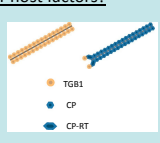


Interaction with nuclear host factors?

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 (not shown).

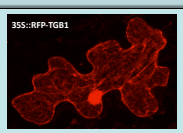
The role of the TGB1 N-terminus in LDM may be related to interactions with host proteins.



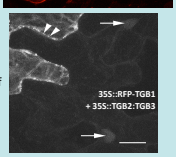
The accumulation of Fluorescent protein-fused TGB1 in the nucleolus could indicate that the native protein shuttles through this compartment to recruit host proteins required for LDM.

35S-expressed TGB1

By itself shows a nuclear and cytoplasmic distribution

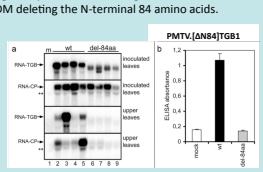


However, co-expression of unlabeled TGB2 and TGB3 from a bicistronic plasmid (35S::TGB2:TGB3), resulting in a ~10:1 TGB2/TGB3 ratio similar to viral infection, resulted in targeting of TGB1 to plasmodesmata (arrowheads). The protein also trafficked intercellularly in the presence of TGB2 and 3 (arrows)



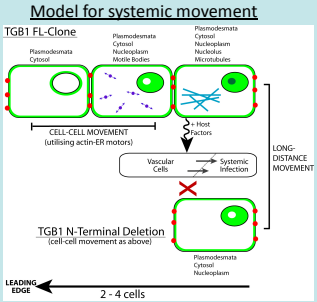
Role of the TGB1 N-terminus in viral long-distance movement

Because fusion of a fluorescent protein to the N-terminus of TGB1 renders the virus incapable of systemic long-distance movement through the phloem, we investigated the role of the N-terminus on LDM deleting the N-terminal 84 amino acids.

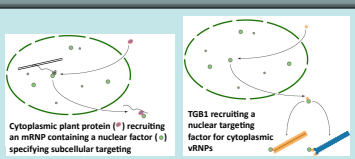


The ΔN84 deletion abolished viral long distance movement, both in the presence and absence of RNA CP

Model for systemic movement



Systemic movement requires entry into the vasculature, which comprises a major barrier [6]. 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.



There are several examples of a nuclear/nucleolar role in subcellular RNP targeting:

1) The umbravirus, Groundnut rosette virus encapsidates its RNA genome with the viral ORF3 gene product in complex with the nucleolar protein fibrillarin, which is recruited by nuclear shuttling of ORF3 [8,9]. We have found that PMTV TGB1 also interacts with fibrillarin (unpublished).

2) Many cellular mRNAs are targeted to specific subcellular locations by specific interactions with RNA binding proteins which become associated with the transcript during splicing [10,11].

3) One nuclear protein involved in cytoplasmic mRNA targeting, VgRBP60, is a homolog of the polypyrimidine tract binding protein (PTB). Intriguingly, a plant PTB homolog has also been shown to be a central part of phloem-mobile RNPs [10,12].

References: [1]Morozov & Solovyev (2003) J Gen Virol 84, 1351-1366; [2]Torrance et al. (2009) MPMI 22, 381-390; [3]McGeachy & Barker (2000) MPMI 13, 125-128; [4]Lim et al. (2008) J Virol 82, 4991-5006; [5]Verchot-Lubicz et al. (2010) MPMI *in press*; [6]Oparka & Turgeon (1999) Plant Cell 11, 739-750; [7]Makarov et al. (2009) J Gen Virol 90, 3022-3032; [8]Kim et al (2007) PNAS 104, 11115-111120; [9]Kim et al. (2007) EMBO J 26, 2169-2179; [10]Farina & Singer (2002) Trends Cell Biol 12, 466-472; [11]Pan et al. (2007) Mol Cell Biol 27, 8340-8351; [12]Ham et al. (2009) Plant Cell 21, 197-215;