Virus proteins take the endocytic path

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Many plant viruses exploit a unique triplet of proteins known as the triple gene block (TGB) of movement proteins to facilitate virus spread and establish a systemic infection in the host plant. The three proteins work together to transport the virus RNA from the initial infected cell, through surrounding cells to the vascular system for translocation throughout the plant. Although some information is available on the relative roles of the TGB proteins, for example TGB1 binds RNA and is thought to form the viral ribonucleoprotein (vRNP) complex, and TGB2 & 3 are integral membrane proteins that assist transportation of the vRNP, the molecular details of the processes are not well understood. We have used a combination of molecular and cell biology tools to try to dissect the mechanisms involved. Two TGB proteins (TGB2 and TGB3) of *Potato mop-top virus* (PMTV) were expressed as N-terminal fusions to green fluorescent protein (GFP) or monomeric red fluorescent protein (mRFP) in epidermal cells of plants. Some of the plants we used were modified so that organelles such as endoplasmic reticulum, actin filaments, microtubules, plasmodesmata and Golgi were labelled with GFP. We used fluorescent markers and chemical inhibitor treatments to investigate the roles of the different organelles and the cytoskeleton

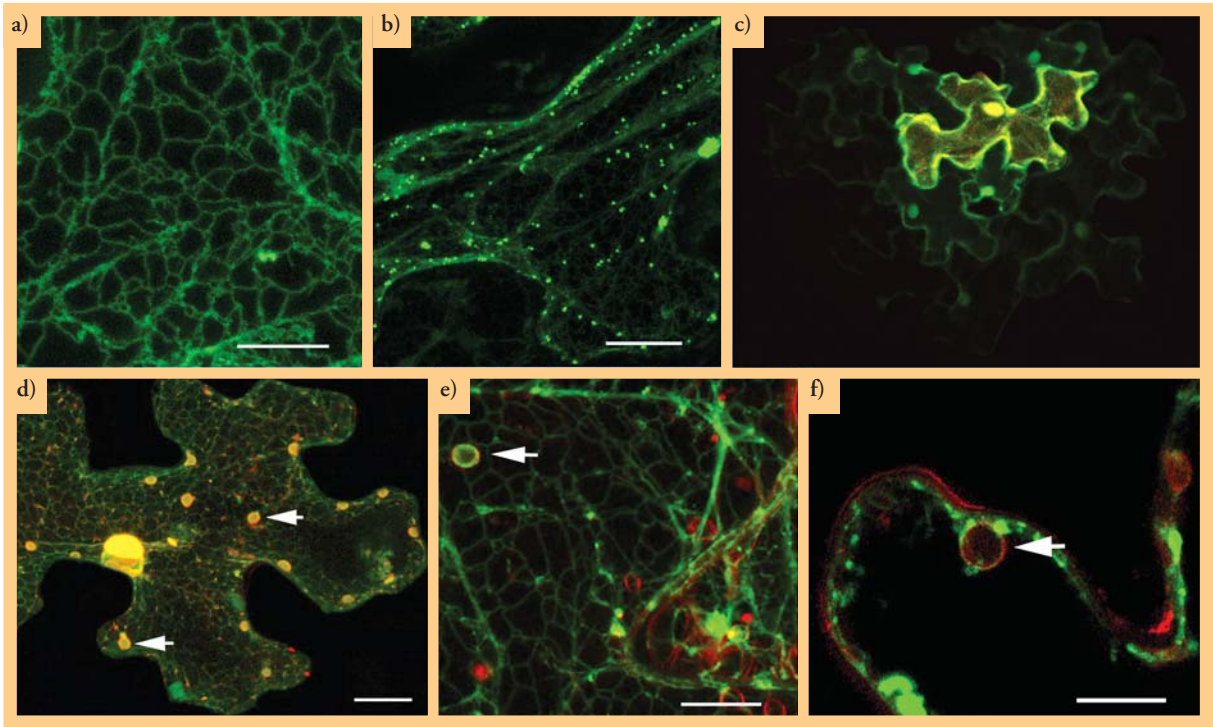

Figure 1 **a)** Transient expression of GFP-TGB2 in epidermal cells of *Nicotiana benthamiana*. Note green fluorescence is prominent in cortical endoplasmic reticulum (ER). Leaves were bombarded with a plasmid expressing GFP-TGB under the control of a 35S promoter (P_{35S}). **b)** Same cells as (a) later in expression; after appearance of green fluorescence in the cortical ER, it is also visible as small motile granules that move quickly at or on the ER network. **c)** Experiments to monitor size exclusion limits (SEL) of plasmodesmata (PD). Leaves were bombarded with a mixture of the plasmids P_{35S}–GFP-sporamin and P_{35S} – mRFP-TGB3. In these and similar experiments with RFP-TGB2, the green fluorescence moved from the initial source cell to neighbouring cells indicating that both TGB proteins increased SEL of PD. No movement of GFP out of source cell was seen in control experiments bombarding cells with P_{35S}–GFP-Sporamin and P_{35S}–mRFP. **d)** Co-localisation of mRFP-TGB2 and GFP-TGB3 in same *N. benthamiana* epidermal cell. Note that GFP-TGB3 appears in ER and together with mRFP-TGB2 in the membranes of the vesicles. At this point in the expression cycle the appearance of mRFP-TGB2 in ER is more patchy. **e)** Co-localisation of FM4-64 and GFP-TGB2; *N. benthamiana* cells were bombarded with P_{35S}–GFP-TGB2 and infiltrated with FM4-64 dye. **f)** Co-localisation of GFP-Ara7 and mRFP-TGB2; *N. benthamiana* cells were co-bombarded with P_{35S}–mRFP-TGB2 and P_{35S}–GFP-Ara7.

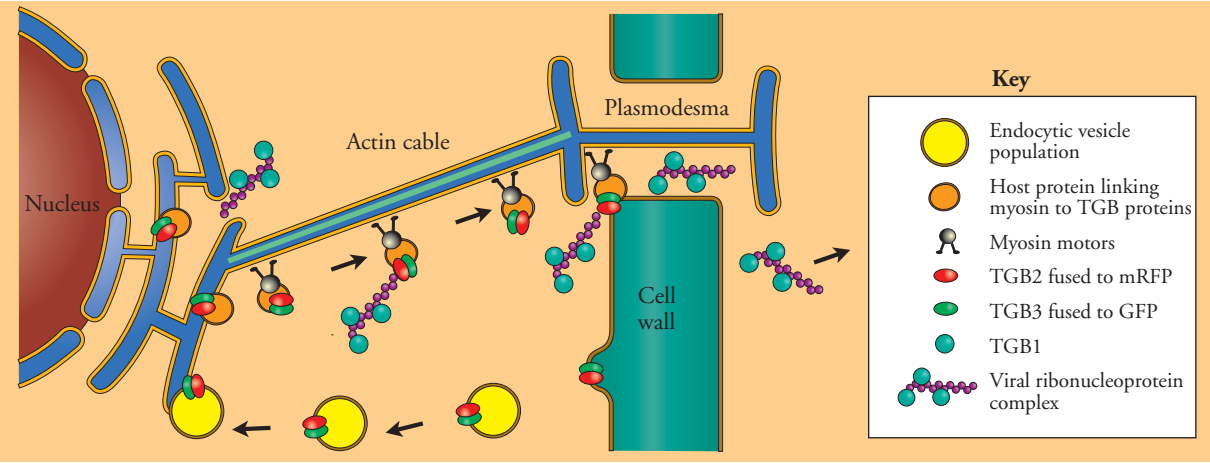

Figure 2 Model indicating the roles of the TGB2 and TGB3 in intracellular movement of PMTV vRNP.

in TGB movement. The fluorescent TGB proteins were visualised in living plant cells using the confocal laser scanning microscope.

These studies revealed that TGB2 and TGB3 fusion proteins associate in small motile granular structures that utilise the endoplasmic reticulum-actin network (Fig. 1a and b) to move quickly to the cell periphery, where they target plasmodesmata. Both TGB2 and TGB3 fusion proteins increased the size exclusion limit of plasmodesmata (Fig. 1c), and fluorophore-labelled TGB3 accumulated at plasmodesmata in the absence of TGB2, suggesting that its primary function is to target plasmodesmata during virus infection. Later, post-targeting of plasmodesmata, both TGB2 and TGB3 fusion proteins were incorporated into vesicular structures (Fig. 1d). TGB2 fusion protein associated with these structures on its own, but the TGB3 fusion protein could not be incorporated into the vesicles in the absence of TGB2. In addition, mRFP-TGB3 was incorporated into vesicles when expressed in PMTV-infected epidermal cells, indicating a cooperation with virus expressed TGB2. FM4-64, a marker for plasma membrane internalisation and components of the endocytic pathway, strongly labelled the TGB fusion protein-containing vesicles (Fig. 1e), while co-localisation of the internalised vesicles with AtRabF2b (Ara7), a Rab5 homologue that marks the early endosome, also implicated the vesicles in the endocytic pathway (Fig. 1f). Protein interaction studies have also revealed that TGB2 interacts with a protein belonging to the RME-8 family of J-domain chaperones, shown recently to be essential for endocytic trafficking in *C. elegans* and *Drosophila*. RME-8 proteins are highly conserved in multicellular organisms from plants to humans, and localise to the limiting membrane of endosomes.

From this work we propose that PMTV TGB2 & 3 associate in membrane bound compartments (probably the small motile granules) and assist transportation of vRNP from sites of RNA replication to the plasmodesmata for transport to neighbouring cells. The TGB2 & 3 do not move from the infected cell but are recycled in the membranes of vesicles derived from the plasma membrane via the endocytic pathway to collect more vRNP (Fig. 2). Future work will aim to show whether the TGB are indeed recycled to the cell interior to resume the trafficking of viral RNA complexes (under control of the virus) or whether they are targeted to the vacuole for subsequent degradation by the plant cell (under control of the host).

References

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