# The role of *Potato mop-top virus* proteins in intracellular movement

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To establish a successful systemic infection, plant viruses must move cell-to-cell from the site of infection to the vascular system where they can be translocated throughout the plant. Intracellular movement involves traversing the infected source cell to reach the plasmodesmata (PD), which are small partially occluded pores in the cell walls. PD are the cells' gatekeepers and regulate the passage of macromolecules, facilitating intercellular communication. Plant viruses have evolved several different strategies to locate and pass through PD, which involve one or more virus-encoded movement proteins (MP). If viruses could not move out of the initial infected cell then the plant would not succumb to infection so a study of movement processes may lead to novel strategies for disease control. The aim of this work is to understand how the movement proteins of *Potato mop-top virus* (PMTV) operate and what host factors are involved in the movement process. This research project is done in collaboration with colleagues in cell-cell communication programme.

The PMTV genome encodes a conserved module of three partially overlapping reading frames called the triple gene block (TGB). We study the expressed proteins using several different experimental systems including biochemical characterisation, transient expression in living cells to examine subcellular localisation, and interactions with host proteins using the yeast two-hybrid system. We have shown previously that the first protein of the movement module (TGBp1) binds RNA and the other two proteins (TGBp2 and TGBp3) are integral membrane proteins that interact with themselves and with each other when expressed in the yeast system. The current model of intracellular movement is that TGBp1 forms a complex with viral RNA (vRNP), which is transported to the periphery of the cell and localised to the PD by interactions with membrane bound TGBp2 and TGBp3. However, the precise details of this process are unknown.

The gene encoding TGBp3 was cloned as a fusion to either green (GFP) or red (RFP) fluorescent protein and transiently expressed in plant cells. A fluorescent network was produced on expression of GFP- or RFP-TGBp3 similar in appearance to the cortical endoplasmic reticulum (ER)(see Figure panel a), fluorescence was also observed associated with the perinuclear envelope and in small punctate spots at the cell periphery suggestive of PD labelling. In addition, TGBp3 formed small spots that could be seen moving along the ER (see Figure panel b), this movement was inhibited by the compound latrunculin A, which is known to depolymerise actin.

To try to identify the structures more accurately, RFP-TGBp3 was transiently expressed on transgenic plants where the ER or PD were labelled with GFP. In these experiments, red fluorescence was seen co-localised to the green ER network (Figure panel c). Also, the peripheral punctate red spots produced by RFP-TGBp3 co-localised to green PD (Figure panel

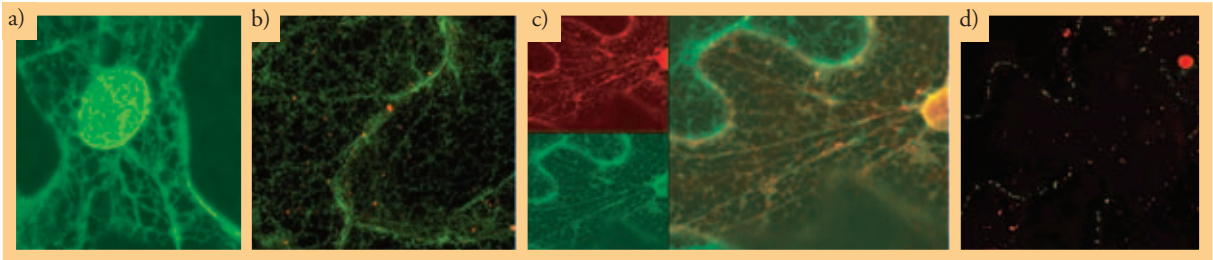


**Figure 1** Confocal microscope images of fluorescent TGBp3 fusion proteins expressed in living cells
a) Network of fluorescence and small motile spots obtained on expression of GFP-TGBp3 in *Nicotiana benthamiana* cells b) Small red fluorescent spots seen moving along ER network in cells of transgenic *N. benthamiana* (where ER labelled with GFP) c) Red fluorescent network co-localises to green ER network when RFP-TGBp3 is expressed on transgenic *N. benthamiana* (where ER labelled with GFP) d) Punctate red fluorescent spots co-localise to green plasmodesmata in transgenic *N. tabacum* (transformed with GFP fused to the 30K MP of *Tobacco mosaic virus*).

d). This data suggests that TGBp3 is involved in transporting the vRNP from the sites of virus replication associated with perinuclear and ER membranes utilizing the cortical ER/actin network. Also, that TGBp3 contains a signal to enable PD targeting, either directly or indirectly via other host factors.

Yeast two-hybrid experiments where TGBp3 was used as bait to search for putative interacting plant proteins have revealed an interaction between TGBp3 and a nuclear envelope protein and further work is in progress to confirm this interaction and understand the role it plays in the movement process. Future work will also examine the role of TGBp2, its coordinate mode of action with TGBp3 and identify and characterise key host proteins and receptors that interact with them.