Multifunctional role of Potato mop-top virus movement protein TGB2

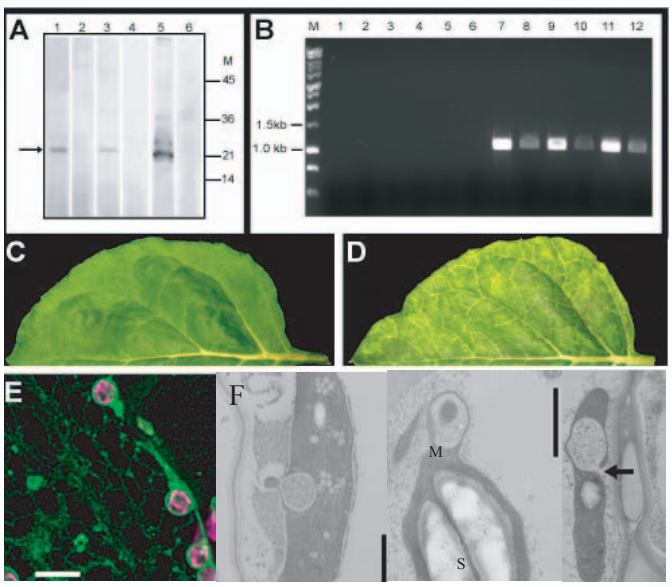
Lesley Torrance, Graham Cowan, Alison Roberts, Sean Chapman, Angelika Ziegler & Eugene Savenkov*. Scottish Crop Research Institute, Invergowrie, DD2 5DA, UK and Swedish University of Agricultural Sciences, Uppsala, Sweden*

contact: ltorra@scri.ac.uk

SUMMARY

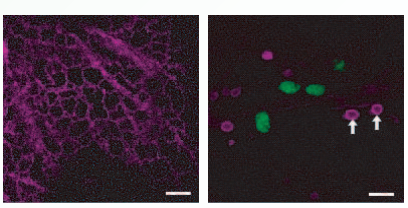
Potato mop-top virus (PMTV) a multi-partite rod-shaped virus encodes three movement proteins known as the triple gene block proteins (TGB). Previous studies on the movement of the integral membrane proteins TGB2 and TGB3 produced the novel finding that they traffick in the endocytic recycling pathway. This paper extends these findings. Expression of monomeric red fluorescent protein (mRFP)-TGB2 in epidermal cells under the control of the 35S promoter or from the PMTV TGB sub-genomic RNA promoter gave identical results. Early in expression association is seen in the ER and moving granules that utilise the actin-ER network to move to the periphery; later it's seen in patches at the plasma membrane, a sub-population of endosomal vesicles and the plastid envelope. Studies using a number of different plant proteins including RabGTPases and markers for Golgi, TGN and endosomes revealed that mRFP-TGB2 co-localised with vesicles implicated in auxin transport. Ongoing research is focused on investigating this novel finding to determine the significance of the interaction.

RESULTS Plastid localisation

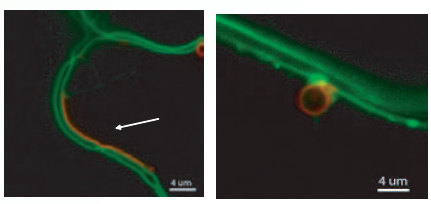


Plastid preparations from PMTV infected leaves tested positive for CP by western blots (A, lanes 1,3,6) and RNA by RT-PCR (B, lanes 7-12). EM examination of thin sections from PMTV-infected leaves with yellow veins (D) but not green tissue (C) showed abnormal shaped chloroplasts with large starch grains and inclusions (F) which were open to cytosol; the inclusions sometimes contained mitochondria. When mRFP-TGB2 was expressed in epidermal cells the fluorescence (E; coloured green) was seen in the chloroplast outer envelope (chlorophyll autofluorecence coloured magenta).

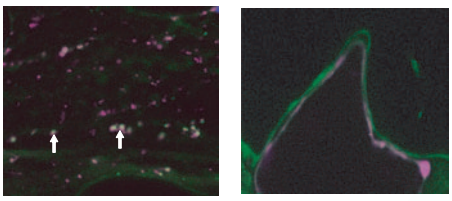
RESULTS Association of mRFP-TGB2 with plasma membrane and endosomes



mRFP-TGB2 localsises to ER, motile granules and small vesicles



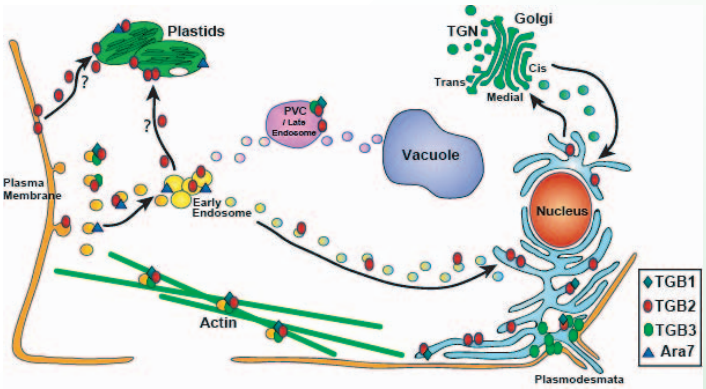
mRFP-TGB2 co-localises to plasma membrane
(transient expression in GFP-PM transgenic plants)



mRFP-TGB2 co-localises in vesicles with GFP-SNX1. PIN2 is transported through SNX1 containing endosomes

mRFP-TGB2 co-localises in peripheral membranes with ARG1 (altered response to gravity)
ARG1 is required for normal root gravitropism and shares vescicular trafficking compartments with PIN auxin efflux carriers

Model of PMTV TGB intracellular trafficking



Virus encoded TGB2 and TGB3 first appear on ER membranes and in ER associated motile granules. They transport the vRNP complexes to the periphery using ER-actin network. TGB3 contains the plasmodesmata targeting signal. TGB2 localises in patches at the PM and contains the signal for endosomal association with the Rab GTPase Ara7 (AtRabF2b), ARG1 and SNX1. Later, TGB2 also localises to the chloroplast envelope with Ara7.
Current hypothesis: TGB2/3 recycle (via PAT endosomes) to reach the ER compartments for rapid transport of vRNP; later there is a switch to chloroplast association and production of genomic RNA.

References and Acknowledgements
Haupt et al (2005) Plant Cell 17, 164; Boonsirichai et al (2003) Plant Cell 15, 2612; Jaillais et al (2006) Nature 443, 106. We thank Thierry Gaude and Patrick Masson for providing the SNX1 and ARG1 materials.