

TSWV particle assembly: *in vivo* interactions between the structural nucleoprotein and G1 spike protein.

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TSWV (Tomato spotted wilt virus) is a member of the Bunyaviridae family. Virus particles are spherical and membrane bound, containing spike proteins that consist of two glycoproteins, G1 and G2. The core contains ribonucleoproteins (RNPs) that consists of genomic RNA tightly associated with the nucleoprotein (N) and small amounts of the viral RNA-dependent RNA polymerase. Enveloped virus particles arise as a result of RNP envelopment with membranes from the Golgi apparatus containing G1 and G2. To investigate *in vivo* interactions between the structural proteins involved in virus assembly, fluorescence techniques (FRET: fluorescence resonance energy transfer and FLIM: fluorescence lifetime imaging microscopy) were employed. To this end, N, G1 and G2 were fused at their N or C-terminus to CFP or YFP. Upon co-expression of N-YFP and N-CFP in mammalian cells, N dimerisation was observed in peri-nuclear aggregates as well as throughout the cytoplasm. The peri-nuclear localisation of N oligomers required actin filaments and microtubules, as demonstrated with the use of inhibitors, which suggested the possible involvement of these cellular elements in TSWV particle formation. Upon co-expression of N-YFP with G2-CFP no FRET was observed, whereas co-expression of N-YFP and G1-CFP did show FRET. Furthermore, G1 was observed to show an altered localisation pattern in the presence of N, i.e. upon single expression of G1 only ER localisation was observed whereas a peri-nuclear accumulation partly overlapping with that of N was observed in the presence of N. Altogether, these results suggest that the actual envelopment of TSWV RNPs' could be triggered by an interaction between N and the cytoplasmic tail of G1.