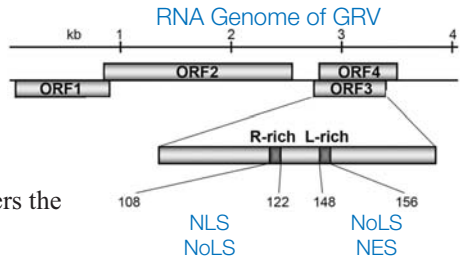
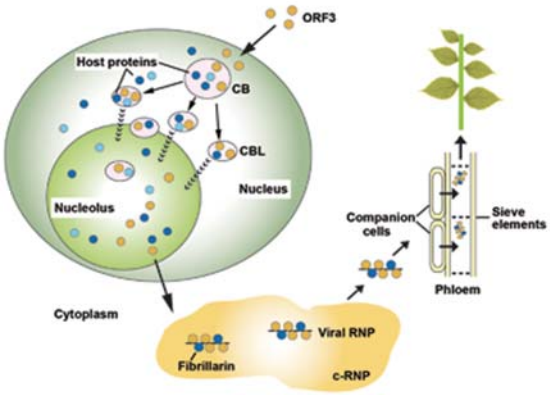
# A Plant Virus Movement Protein Forms Ringlike Complexes with the Major Nucleolar Protein, Fibrillarin, *In Vitro*

**Jane Shaw**[1], Elisabetta Canetta[2], Sang-Hyon Kim[1], Natalia O. Kalinina[1], Ashok Adya[2], John W. S. Brown[1] and Michael Taliansky[1]
[1]Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA, UK.
[2]School of Contemporary Sciences, University of Abertay, Dundee, Bell Street, Dundee, DD1 1HG, UK.

## Umbravirus ORF3 is required for virus systemic movement

Umbraviruses such as GRV and PEMV do not encode a conventional coat protein and thus do not form virus particles. Instead, the ORF3 protein interacts with the viral RNA *in vivo* to form filamentous RNP particles and facilitates the long distance movement of the virus. The sequence of ORF3 contains 2 conserved regions, an argine and leucine-rich region (R and L) which act as nuclear/nucleolar localisation and export signals.




ORF3 is produced in the cytoplasm, enters the nucleus and targets Cajal bodies (CBs).

This leads to rearrangement of CBs into multiple CB-like structures (CBLs).

CBLs contains the host protein fibrillarin and they fuse with the nucleolus.

The ORF3 and fibrillarin relocate to the cytoplasm where RNP complexes containing viral RNA, ORF3 and fibrillarin accumulate.

If these RNP complexes are produced in companion cells, they have the ability to move into the vasculature of the plant leading to systemic infection.

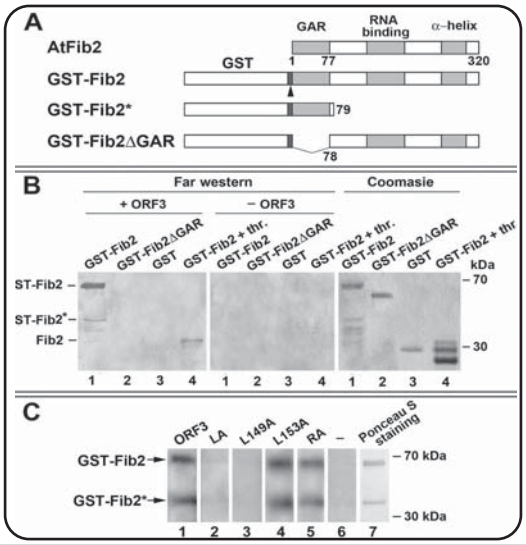
(The EMBO Journal (2007), **26**, 2169; PNAS (2007) **104**, 11115)

## ORF3 interacts with fibrillarin

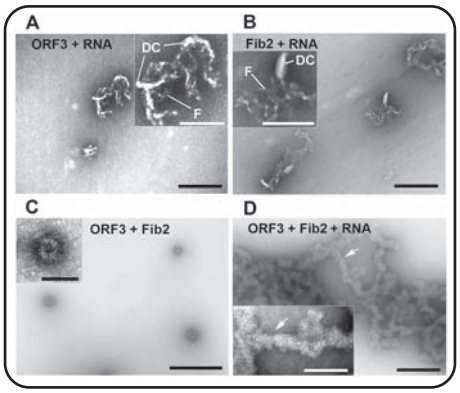
Fibrillarin is the major nucleolar protein and is a core component of small nucleolar ribonucleoprotein particles (snoRNPs) and is required for rRNA processing. The protein contains various domains including the glycine and arginine-rich (GAR) domain, an RNA binding domain and an α-helix.

Using GST-Fibrillarin fusions, it was determined from Western analysis that the ORF3 protein of GRV interacts directly with the GAR domain of fibrillarin *in vitro*.

Analysis of mutations in the R- and L-rich regions of ORF3 not only interrupted nuclear/nucleolar localisation but in addition is was concluded that the L-rich domain is directly involved in the interaction with fibrillarin.



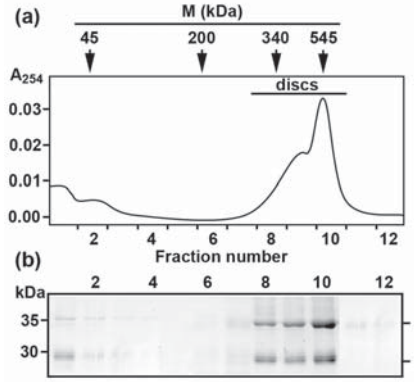

## Fibrillarin mediates assembly of viral RNPs



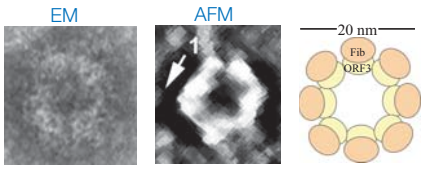

ORF3 binds umbraviral RNA *in vitro* to form different structures to viral RNP particles formed *in vivo* (A). Viral RNA mixed with fibrillarin forms complexes similar to ORF3+RNA, a mixture of thin (F) and thicker filaments (DC) (B). Incubation of ORF3+fibrillarin+viral RNA leads to the formation of regular filamentous structures similar to *in vivo* RNP particles (D). When ORF3 and fibrillarin are incubated together, disc-like complexes of 18-22 nm are formed.

## ORF3 and fibrillarin form ring-like complexes

ORF3, fibrillarin and complexes formed by incubating ORF3 and fibrillarin together *in vitro* were anlaysed using electron microscopy (EM) and atomic force microscopy (AFM). Fibrillarin molecules were observed as granules of height ~1.9 nm in diameter by ~10 nm in height and represent monomers. 3 different classes of ORF3 particles were observed (~1.6 nm, ~3.1 nm and ~4.2 nm) suggesting ORF3 forms multimers.




**Mixing ORF3 with fibrillarin leads to the formation of ring-like complexes of 18-22 nm in diameter in a 1:1 ratio.**




References : The EMBO Journal (2007) **26**, 2169; PNAS (2007) **104**, 11115; J. Mol. Biol. (2008) **376**, 932.