Involvement of nucleolus in plant virus infection

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The past few years have brought remarkable progress in our understanding of the genome organization and expression of umbraviruses (Taliansky and Robinson, 2003). At the same time, the recent findings raised some new and fascinating questions related to basic molecular processes in plants. Involvement of the nucleolus in umbravirus infection is among them.

The nucleolus is a prominent subnuclear domain and is classically regarded as the site of transcription of rRNA, processing of the pre-rRNAs and biogenesis of pre-ribosomal particles. However, in addition to these 'traditional' nucleolar activities, the nucleolus also participates in many other aspects of cell function. Thus, because of sequestration and maturation of several factors and regulatory complexes, the nucleolus is involved in the regulation of signal recognition particle biogenesis, small nuclear RNA processing, mRNA nuclear export, telomerase activity, the cell cycle, cell growth and aging.

Umbravirus-encoded ORF3 protein is a multifunctional RNA-binding protein involved in phloem-associated long-distance movement of viral RNA, and its protection from RNase attack (Ryabov *et al.,* 1999; 2001 see also *SCRI Ann. Rep. 1999/2000, 144-146*). Localization studies showed that the ORF3 protein encoded by *Groundnut rosette virus* (GRV, an umbravirus) accumulated in cytoplasmic granules. These granules consisted of filamentous ribonucleoprotein (RNP) particles, contained viral RNA and the ORF3 protein. It is suggested that these RNP particles serve to protect viral RNA, and may be the form in which it moves through the phloem. (Taliansky *et al.*, 2003). Formation of the cytoplasmic RNP complexes may also be involved in the protection of viral RNA from the plant's defensive RNA silencing response. Consistent with this suggestion, heterologous expression of the ORF3 protein in cultured *Drosophila* cells led to formation of similar particles in the cytoplasm of the cells. The RNA content of these particles produced in *Drosophila* cells is not presently known but expression of the ORF3 protein led to suppression of RNA interference in the *Drosophila* cells.

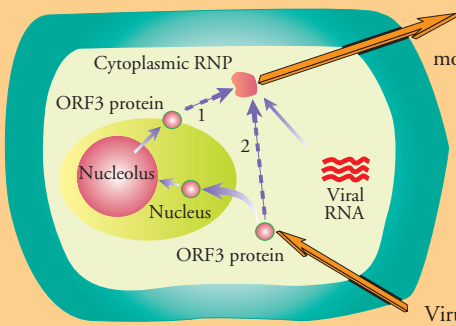

Figure 1 Schematic diagram of the pathway taken by umbraviral ORF3 protein in an infected cell. It is unclear whether the protein reaches the cytoplasmic RNP complexes from the nucleus/nucleolus (1), or directly through the cytoplasm (2).

The studies of localization of the ORF3 protein also provided another quite unexpected finding; in addition to the cytoplasmic granules, the ORF3 protein was also found in nuclei, preferentially targeting nucleoli. Comparison of amino acid sequences of the umbraviral ORF3 proteins revealed two highly conserved domains one of which included an R-rich sequence and another one contained invariant L residues. Alanine scanning mutagenesis showed that both the R-rich and L-rich domains were involved in the localization of the ORF3 protein to the nucleolus. In addition, the L-rich domain also functioned as a nuclear export signal (NES), suggesting that the ORF3 protein is a nuclear/nucleolar shuttle protein. Functional analysis of the mutants revealed the correlation between the ORF3 protein nucleolar localization and its ability to transport viral RNA long distances *via* the phloem. The likely pathways taken by ORF3 protein in infected plant cell is illustrated in Fig. 1. This suggests that the nucleolar functions could be involved in the process of long-distance RNA movement and possibly protection of RNA from RNA silencing.

How and why does the umbravirus protein modify nucleolar activities? Does it interact with RNA components of the nucleolus, such as rRNAs or small nucleolar RNAs to modify RNA metabolism or does it bind to one or more nucleolar proteins, inhibiting their enzymatic or other activities? Does the ORF3 protein have an effect on nucleolar sequestration of the cell growth and cell cycle regulators? Future research will address these questions and attempt to open up the "nucleolar black box" in our understanding of the functional links between nucleolar functions and long-distance virus (and more generally, macromolecular) transport in plants.