propagation in cell culture. In addition, overexpression of E1 and E2 proteins was able to increase the propagation rate. Propagation was not mediated by fusion of infected with non-infected cells, and cell-to-cell contacts did not seem to be essential for this process. Instead, cells infected or transfected with SFV devoid of capsid were able to release infectious microvesicles which contained envelope proteins at their surface and viral RNA inside. These microvesicles appeared to infect cells following a process that also required an acidic pH in endosomes. In order to evaluate the possibility of using these infectious membranous particles as expression vectors, the GFP gene was cloned into the SFV genome devoid of capsid downstream of a second subgenomic promoter. This vector was able to transfer GFP expression from initially infected or transfected cells to most cells in a culture after a few hours. Although these microvesicles were produced at low levels, this minimalist infectious system could impact the way we see viruses today. In fact, they could mimic some sort of primordial envelope viral systems that had not yet acquired a nucleocapsid able to efficiently package and deliver the viral genome.

Keywords: alphavirus, capsid, envelope proteins, budding.

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Role of the 5' untranslated region of the Alfalfa mosaic virus RNA 3 in cell-to-cell and long distance transport

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After the start of the infection in a single cell, plant viruses need to invade the adjacent cells, a process denominated cell-to-cell transport, as a previous step to invade the distal parts of the host through the vascular system or systemic transport. The capacity to reach the uninoculated parts of a plant implies that the virus should infect specific cells located at the vascular tissue. In most cases, virus particles are required for this vascular transport. In the present study we have addressed the characterization of viral determinants critical for the long distance transport using the *Alfalfa mosaic virus* (AMV) model system, which requires virus particle for the systemic transport.

AMV is the type member of the Alfamovirus genus within the family *Bromoviridae*. Its genome consists of three positive RNAs. Monocistronic RNAs 1 and 2 encode P1 and P2 proteins of the RNA polymerase complex, respectively. RNA 3 contains two open reading frames encoding the movement protein (MP) and coat protein (CP), which is expressed through a subgenomic RNA or RNA 4.

Previous analysis showed that the AMV MP gene is functionally interchangeable for long and short distance transport by the corresponding gene of viruses belonging to eight genera of the viral family 30K (Sanchez-Navarro et al.; 2006 Virology 341: 66-73; Sánchez-Navarro et al., 2010, J. Virology 84: 4109-4112). However, the exchange of the *Brome mosaic virus* MP lacking the C-terminal 48 amino acid residues, generated a chimeric RNA 3 (MPBMV255/CP) defective for long-distance transport (Sánchez-Navarro et al., 2001; MPMI 14: 1051-1062). In this study we performed viral evolution experiments, addressed to characterize RNA 3 determinants of the chimera MPBMV255 / CP critical for systemic transport. After the seventh passage, we observed systemic infection in all lines. The analysis of the nucleotide sequence revealed that all RNA 3 variants present in the upper parts of the plants contained deletions at the 5' untranslated region (5' UTR). Further analysis of the evolved 5'UTR revealed that this region, in spite of reducing the expression of the MP and drastically the encapsidation of the viral progeny, incremented the cell-to-cell transport. Interestingly, we observed that the modified 5'UTR permits the systemic transport of an AMV variant defective in virus particles formation. The evolutive implications of these observations for the cell-to-cell and systemic transport of plant viruses will be discussed.

Keywords: Systemic transport; 30K family; Alfamovirus; virus evolution.

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Structural studies of PRD1 genome delivery device

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PRD1 is an internal membrane-containing bacteriophage that infects Gram-negative cells. A wealth of biochemical and structural information has been accumulated on PRD1 during the past 20 years. So far, it remains the only phage with a membrane whose icosahedral structure has have been visualized at 4.2Å by X-ray crystallography elucidating fundamental aspects of viral evolution. To deliver its double-stranded DNA, the icosahedral protein-rich virus membrane transforms into a tubular structure protruding from one of the twelve vertices of the capsid.

Here, using a combination of electron microscopy techniques, we study PRD1, the best understood model for lipid-containing viruses, to unveil the mechanism behind the genome translocation across the cell envelope.