Annotation of Acyrthosiphon pisum genes potentially involved in Luteoviridae transcytosis: yeast two-hybrid system to confirm interaction between aphid and virus proteins

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The Luteoviridae family is composed of single-stranded RNA plant viruses restricted to phloem tissues that are specifically transmitted by aphids in a circulative, non-propagative manner. In order to be successfully transmitted, luteovirids (members of the *Luteovirus* and *Polerovirus* genera) must cross two epithelial "barriers": (i) the midgut and/or hindgut cell layers to enter insect haemocoel and (ii) the accessory salivary gland cell layer to be inoculated to a healthy plant through saliva. Transcytosis of these cell layers relies on the presence of specific aphid components (receptors) able to recognize structural viral proteins and then to sustain virion transport (endocytic components) from one pole to the other in the cell (Gildow 1999; Brault *et al.,* 2007). Transmissible virions enter the cells following a clathrin-mediated endocytosis (CME) process and are transported across the cells enclosed in different types of vesicles (Gildow, 1993). It is believed that the virions hijack a naturally occurring mechanism which most likely enables transport of essential aphid macromolecules (Marsh & Helenius, 2006).

Acyrthosiphum pisum (Harris) is the insect vector of two luteoviriduses: *Pea enation mosaic virus* (PEMV) and *Soybean dwarf virus* (SbDV). However, the virus pathway in this aphid is not as completely described as in the case of other aphid/luteovirid combinations (Brault *et al.*, 2007) like *Myzus persicae* (Sulzer) / *Beet western yellows virus* (BWYV) for example.

Accordingly, we have annotated 45 *A. pisum* genes implicated in clathrin-mediated endocytosis, using previously identified members of the CME pathway in *Drosophila melanogaster* Meigen and occasionally in *Homo sapiens* L. or *Rattus norvegicus* Berkenhout. This finding represents a quasi-perfect correlation with *D. melanogaster* and is in good agreement with previous reports affirming the conservation of the CME network across Animalia (Schmid & McMahon, 2007).

Moreover, we have annotated some genes or families of genes previously identified as implicated in luteovirid transmission (Seddas *et al.,* 2004; Yang *et al.*, 2008): Rack-1 (Receptor for activated C kinase 1; 1 gene), Gapdh (Glyceraldehyde-3-phosphate dehydrogenase; 4 genes), luciferase (Acyl-CoA synthetase; 11 genes) and cyclophilin (peptidyl-prolyl cis-trans isomerase, 14 genes).

In order to confirm interaction between some of these candidates (Rack-1 and Gapdh), we developed a yeast 2 hybrid (Y2H) system using structural viral proteins (major and minor capsid protein) as bait and each *M. persicae* candidate as prey.

In order to identify new candidates ("membrane receptors") of structural BWYV proteins we will employ a split-ubiquitin Y2H system coupled with an accessory salivary glands library and a digestive tract cDNA library which we will make from *M. persicae.*

Key words: Aphids, luteovirus, circulative transmission, clathrin-mediated endocytosis, membrane receptor

BRAULT V., HERRBACH E., REINBOLD, C., 2007. Electron microscopy studies on luteovirid transmission by aphids. Micron, 38: 302-312.

GILDOW F., 1993. Evidence for receptor-mediated endocytosis regulating luteovirus acquisition by aphids. Phytopathology, 83: 270-277.

GILDOW F., 1999. Luteovirus transmission mechanisms regulating vector specificity. In: The Luteoviridae (eds Smith H.G., Barker, H.). CABI, Wallingford, pp. 88-111.

MARSH M., HELENIUS A., 2006. Virus entry: open sesame. Cell, 124: 729-740.

SCHMID E.M., MCMAHON H.T., 2007. Integrating molecular and network biology to decode endocytosis. Nature, 448: 883-888.

SEDDAS P., BOISSINOT, S., STRUB J.M., VAN DORSSELAER A., VAN REGENMORTEL M.H., PATTUS F., 2004. Rack-1, GAPDH3, and actin: proteins of *Myzus persicae* potentially involved in the transcytosis of beet western yellows virus particles in the aphid. Virology, 325: 399-412.

YANG X., THANNHAUSER T.W., BURROWS M., COX-FOSTER D., GILDOW F.E., GRAY S.M., 2008. Coupling genetics and proteomics to identify aphid proteins associated with vector-specific transmission of polerovirus (luteoviridae). Journal of Virology. 82: 291-299.