-DESVENDANDO O ENVOLVIMENTO DA PROTEÍNA P32 DOS CILEVIRUS NO TRANSPORTE VIRAL

UNRAVELLING THE INVOLVEMENT OF CILEVIRUS P32 PROTEIN IN THE VIRAL TRANSPORT

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Resumo:

Introduction. Citrus leprosis (CL) is a severe disease that affects citrus orchards mainly in Latin America. It is caused by *Brevipalpus*-transmitted viruses from genera *Cilevirus* and *Dichorhavirus*. Currently, no reports have explored the movement machinery for the cilevirus. **Objective.** The aim of the present study was to evaluate the functionality of the p32 protein of cileviruses. Here, we have performed a detailed functional study of the p32 movement protein (MP) of two cileviruses, *citrus leprosis virus C* (CiLV-C) and *citrus leprosis virus C2* (CiLV-C2). **Methodology and Results.** Citrus leprosis-associated viruses are not able to move systemically in neither their natural nor experimental host plants. However, here we show, by detection of infection foci in transgenic tobacco P12 plants, that cilevirus MPs are able to allow the cell-to-cell and long-distance transport of movement-defective alfalfa mosaic virus (AMV). The cis-expression of the wild-type and truncated versions of the MPs of two cilevirus, by inoculation of viral RNA transcripts on P12 leaves, using different versions of a movement defective AMV clone, revealed several features related with the viral transport, including: i) the ability of cilevirus MPs to facilitate virus movement on a nucleocapsid assembly independent-manner; ii) the generation of tubular structures from transient expression in protoplast and; iii) the role of the C-terminus of p32 in the cell-to-cell and long-distance transport, tubule formation and the MP-plasmodesmata co-localization. Using the bimolecular fluorescence complementation (BiFC) method and co-infiltration assay in *Nicotiana benthamiana*, we observed the capability of the N- and C- terminus of MP to interact with the cognate capsid protein (p29), and the MP's ability to direct the p29 to the plasmodesmata, whereby the C-terminus of MP is independently responsible to recruit the p29 to the cell periphery. Furthermore, from *in vivo* BiFC and co-immunoprecipitation (Co-IP) analysis, we report that MP possess the capacity to enter the nucleolus and to bind to a major nucleolar protein, the fibrillarin. **Conclusion**. Based on our findings, we elucidate the movement functionality of the cileviruses p32 protein, and provide a model for the role of the p32 in the intra- and intercellular viral spread.

Palavras-chave: Leprosis-associated viruses; *Cilevirus*; Cell-to-cell and systemic movement; AMV model system; Fibrillarin (Fib2)

Apoio

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