Visualization of virulence protein translocation from *Agrobacterium* to yeast and plant cells.

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During *Agrobacterium*-mediated transformation of eukaryotic organisms a number of effector proteins (VirD2, VirD5, VirE2, VirE3 and VirF) are translocated from the bacterium into the host cell. The VirE2 protein is an essential effector protein for the transformation of plant cells. After translocation, it binds to the T-DNA without any sequence specificity to protect it from host nucleases. Besides, it is required for T-DNA delivery to the nuclei of plant cells. However, the translocation process itself and the fate of translocated VirE2 inside the recipient cell are poorly understood. In this study, we used the split-GFP strategy for visualization of the translocation of VirE2 to both plant and yeast cells. To this end, we co cultivated *Agrobacterium* strains expressing VirE2 N-terminally and internally tagged with GFP11 with host cells expressing the complementary part of GFP, GFP1-10. Already after 8 hours of co-cultivation fluorescent filamentous and dot-like structures were visible in both *Saccharomyces cerevisiae*, *Arabidopsis thaliana* Col-0 and *Nicotiana tabacum* SR1 cells. Similar results were obtained when *Agrobacterium* strains lacking T-DNA were used. Expressed tagged VirE2 as a filament colocalized with microtubules and dot-like spots aggregated at the spindle poles in dividing cells (1). Using a similar approach we showed that other virulence proteins were translocated to yeast cells. The localization was comparable when *Agrobacterium* strains lacking T-DNA were used.

(1) Sakalis, P.A., van Heusden, G.P.H. & Hooykaas, P.J.J., 2014. Visualization of VirE2 protein translocation by the Agrobacterium type IV secretion system into host cells. MicrobiologyOpen, 3, 104–117.