Evidence of a Novel Citrus Protein Complex Involved in Lys63-linked Polyubiquitylation of a *Xanthomonas* Type III Effector

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Citrus canker, caused by the bacterial pathogen *Xanthomonas axonopodis* pv. *citri* (Xac), is considered a major threat to the world citriculture. During infection, Xac delivers a number of effector proteins into the plant cell via a type III secretion system, including members of the AvrBs3/PthA protein family. PthA proteins are thought to manipulate the transcription of the host genes leading to hypertrophy and hyperplasia of the host cells. To elucidate how PthA activates transcription and to establish its molecular mode of action, yeast two-hybrid assays were employed to identify citrus proteins that interact with such effector. Here, we present evidence for the interaction between each of the four variants of PthA proteins with the citrus Hip-Trx (Hsp70-interacting protein-thioredoxin), Cyclophilin, Uev (Ubiquitin conjugating enzyme E2 variant), Ubc13 and alpha-importin, involved in protein folding, ubiquitylation and nuclear import, respectively. Interestingly, the specificity of the interactions appears to depend on the internal repetitive and variable domain of the PthA proteins. Most significantly though, we found that the citrus proteins Hip-Trx, Cyclophilin, Uev and the Ubc13 interact to each other, given support for the existence of a new chaperone complex implicated in folding/processing and ubiquitylation of PthAs. This is consistent with the notion that effector proteins delivered by the type III secretion system enter the host cell in an unfolded or inactive state.

Key words: PthA/AvrBs3, *Xanthomonas citri*, folding/ubiquitylation complex, Hip-Trx, Cyclophilin, Ubc13-Uev.

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