

The virulence factors CoNpc1 and CoNpc2 are required for intracellular sterol transport and appressorial penetration of *Colletotrichum orbiculare*

Sayo Kodama, Naoki Kajikawa, Fumi Fukada, Yasuyuki Kubo

Graduate School of Life and Environmental Sciences, Kyoto Prefectural University, Japan

Purpose and methods: Fungal morphogenesis depends on accurate cell cycle progression. GTPase activating protein complex CoBub2-CoBfa1 interacts with a downstream factor, GTPase CoTem1, and is required for G1/S progression and pathogenesis in the cucumber anthracnose fungus *Colletotrichum orbiculare*. To elucidate the signal cascade of CoTem1, we screened physical interaction factors with CoTem1 by Yeast Two-Hybrid system and identified a phosphatidylglycerol phosphatidylinositol transfer protein CoNpc2. The Niemann-Pick type C (NPC) proteins NPC1 and NPC2 are sterol-binding proteins required for the export of lipoprotein-derived sterol from lysosomes in mammals.

Results: The CoNpc2 co-localized with CoNpc1 and a late endosome marker CoRab7 at vacuoles and granular body. Furthermore, sterol stained by filipin III was observed along the conidial and appressorium membrane in the wild type, whereas filipin staining in *conpc1* and *conpc2* mutants was recognized in the conidial vacuole, suggesting that NPC proteins are involved in sterol transport in *C. orbiculare*. The *conpc1* and *conpc2* mutants formed normal appressoria, however, penetration hyphae were not observed, thus the lesion formation of the host plant was markedly reduced. By contrast, the *conpc1* and *conpc2* mutants formed lesions similar to the wild type on wounded cucumber leaves, suggesting that NPC proteins are not required for invasive growth. Furthermore, TEM observation revealed immature appressorial cone of *conpc2* mutant that lead to the defect in penetration peg formation on host plant surface.

Conclusion: Taken together, CoNpc1 and CoNpc2 function as a sterol transporter required for appressorium-mediated host cuticle penetration.